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Dietary change strategies for sustainable diets and their impact on human health, volume 1

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Dietary change strategies for sustainable diets and their impact on human health - volume 1

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A Food System Approach for Sustainable Food-Based Dietary Guidelines: An Exploratory Scenario Study on Dutch Animal Food Products

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This study explores interconnections between food consumption and production of animal (by-)products in different food system scenarios within the scope of Dutch Food-Based Dietary Guidelines (FBDG). For this scenario study, a Microsoft Excel model was created that include seven scenarios with different quantities of eggs, milk, cheese, beef cattle, broilers, and pigs as input. Number of animals, intake of energy, animal protein, saturated fatty acids (SFAs), trans-fatty acids (TFAs), salt, greenhouse gas emissions (GHGEs), and land use (LU) were calculated and compared with current consumption and reference values. Based on the concept of eating the whole animal, every recommended lean, unprocessed portion of beef comes along with a non-recommended portion of beef (two portions for pork, 0.5 portion for broilers). The reference values for SFAs, TFAs, and salt were not exceeded if the intake of meat is limited to 410 g/week. The scenarios with recommended 450 mL semi-skimmed milk and 40 g/day low-fat cheese results in 36 g/day of butter as by-product, exceeding its acceptable intake three times. The near-vegetarian scenario with recommended amounts of eggs, milk, and cheese, includes only a portion of beef/calf per 6 days and a portion of chicken per 9 weeks as by-products. This scenario more than halves the GHGE and LU. Finally, the scenario that included the maximum recommended amounts of animal products is reachable with half the current size of Dutch livestock. This conceptual framework may be useful in the discussion on how future sustainable FBDG can incorporate a more food system-based approach.

Keywords: food system scenarios, sustainable food-based dietary guidelines, animal food products, environmental impact, healthy diets, livestock size

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INTRODUCTION

FBDG Should Be Sustainable

Worldwide, governments have agreed to prioritise actions toward sustainable development in line with the United Nations Sustainable Development Goals (1). Food systems are highly relevant for this sustainable development (2, 3). It is widely acknowledged that current food systems are not ecologically sustainable. Food production and consumption are not within the so-called safe operating space, thereby not complying to ecological nor health objectives (4). In fact, food production and consumption are two of the main drivers of global climate change (3, 5). Concerns about ecological boundaries will only increase further if no steps are taken toward more sustainable food systems globally (6). Although there has been increased focus on this topic in recent years, many gaps in the knowledge of the relationship among environmental factors, food systems, and nutritional outcomes persist (7).

Current Diets and Recommendations

At present, global food production and consumption have a share of 21–37% in total greenhouse gas emissions (GHGEs) and up to 40% in land use (LU) (8). The same is true for the Dutch consumption (9). Animal (-derived) food products such as red and white meat, milk products, and cheese are the largest contributors to the ecological impact of the current diet (10). In the Netherlands, animal (-derived) foods together contribute to about 60% of the total diet-related GHGEs. However, the present calculations concerning ecological impact underlying the Food-Based Dietary Guidelines (FBDG) are based on individual food products and their consumption rather than interrelated products within a closed food system [e.g., (11)].

The Federation of European Nutrition Sciences established a task force for developing a conceptual framework for the future development of FBDG in Europe. One of the conclusions was that environmental aspects should be included in the future conceptual framework for FBDG (12). In addition, a further study needs to be done exploring current practises, existing methodologies, and the prospects for incorporating other relevant dimensions into a future conceptual framework for Sustainable FBDG in Europe (12). Also, the Food and Agriculture Organisation (FAO) and WHO embrace the concept of sustainable dietary guidelines. They developed guiding principles around what constitutes sustainable healthy diets, to be further translated into clear, non-technical information and messages to be used by governments and other actors in policy-making and communications (13). Several European countries have already taken some dimensions of sustainability into account in their most recent FBDG (14), which include some ecological perspectives in The Netherlands (15). Nevertheless, only a slight reduction in the dietary impact of the Dutch diet on GHGE and LU will be achieved if the current maximum amount of recommended meat (500 g/week) would be consumed (11). The GHGE of the Dutch guidelines are relatively high compared with those of other countries (16), so there is a need for further development of FBDG in the direction of sustainability.

Dependencies in the Food System

A knowledge gap exists on the ecological impact of Dutch FBDG from a food system perspective. Typically, research on how to achieve more sustainable diets has focussed on two ways. First, the production pathway in which reducing the ecological footprint of animal (-derived) products per kg of product is emphasised [e.g., use of feed from waste streams (17)]. Second, the consumption pathway, which focuses on eating less or no animal (-derived) products, for instance, switching to a vegetarian or vegan diet (18). However, neither of these approaches consider the elements as operating in one food system (19). The analysis of Springmann et al. (20) suggests that national guidelines could be both healthier and more sustainable by providing clearer advice on limiting, in most contexts, the consumption of animal source foods, in particular beef and dairy. Therefore, this study focuses on animal food products.

Questions that arise when adopting a food system approach are, for instance: could the ecological impact of the FBDG be reduced by consuming roosters (vs. only broilers) as a source of chicken meat, or by consuming meat from laying hens and/or dairy cows and their calves? Could the ecological impact of the FBDG be reduced by consuming all parts of the animal instead of only lean cuts? And what would be the nutritional consequences? Despite the substantial evidence showing the need and possibilities for aligning health and environmental objectives, only a minority of countries have, so far, included environmental sustainability in their FBDG, but none with a food system approach (21). In fact, the majority of research on sustainable diets tends to focus on individual food products rather than products within a food system (22). The focus of this article is an exploratory scenario study on Dutch animal food products in relation to the Dutch FBDG. The Dutch food system and guidelines are comparable with those of several other northern European countries such as Belgium, Germany, United Kingdom, and Nordic countries. Therefore, the objective of this study is to explore different food system scenarios, ultimately to investigate the implications of the results on future sustainable FBDG in the Netherlands. Thereby, the focus will be on a closed loop of food grade (safe for human consumption) animal (by-) products between production and consumption on a national level.

METHODS

For this investigation, we conducted an exploratory scenario study on Dutch animal food products and interdependencies in the animal food system using a model built in Microsoft Excel.

Data Collection

A data set in Excel was created that integrates both extensive information on the livestock and its by-products and interdependencies, and information on the production and consumption of livestock production systems in the Netherlands. The studied livestock systems are laying hens and broilers, dairy and beef cattle, veal cattle, and pigs.

For each livestock production system, the model includes:

- The animal components (see Section Building the Model) of the system and their interdependencies, such as portion weight and ratios between different components of each livestock production system
- The nutritional value (energy, animal protein, SFA, TFA, salt) of each component, and the separation in edible and marketable meat and dairy parts
- The current nutritional advice regarding these components according to the Dutch FBDG

- Categorisation of the components in or outside the Wheel of Five (classification is explained in Section Nutritional Classification of Animal Products)
- The ecological impact (GHGE and LU) of each component, including processing and transport
- Consumption and production statistics of each component.

For nutritional data of the components, the Dutch Branded Food Database was used (23). Regarding production data, mainly the open data source StatLine from Statistics Netherlands CBS was used (24, 25). An exception was production data of dairy: these were extracted from ZuivelNL, because they were more detailed (26). Consumption data were extracted from the Dutch National Food Consumption Survey 2012–2016 (27). The LCA database of the RIVM was used (28), such as the extrapolated data for the RIVM healthy diets study (11). The GHGE and LU data are generated by life cycle analysis according to the Agro-Footprint method (29). Missing data were taken from data sources by Blonk Consultants (30). We selected GHGE and LU as they cover most of the diet-related ecological impact. Although nitrogen emission through manure and ammonia is an important ecological impact in the livestock production system, we lack data on the nitrogen footprint per product (31).

Data required to calculate ratios between the components of the livestock production systems (productivity, yield, edible weight) were taken from different sources:

- productivity overall: Blonk Consultants or Blonk Agri-footprint BV (26, 29, 32),
- dairy system: ZuivelNL (26) (dairy producer platform),
- chicken system: Plukon Food Group, Kipster, www.kipinnederland.nl (association of Dutch poultry producing firms) and
- beef and pork system: SVH, www.vlees.nl (meat producer platform).

The quantities of edible weight per animal are based on Luske and Blonk (32). The sources were selected after consultation with three experts in this field.

Nutritional Classification of Animal Products

Currently, the Dutch FBDG are set up in the form of a nutritional education model: The Wheel of Five, further referred to as "the Wheel" [see **Figure 1**; the five groups; bread, grains, and potatoes; drinks; fruits and vegetables; fats; and dairy, nuts, fish, legumes, meat, and eggs; (33)]. The Wheel models an optimal combination of food product groups that both maximises health benefits and satisfies nutritional needs according to the recommendations of the Health Council of the Netherlands (34, 35). The general advice is to eat especially food products included in the Wheel (divided over five main categories) and to limit the consumption of products that fall outside the Wheel (divided in the categories "Daily choice" and "Weekly choice"), explained in more detail elsewhere (21). One of the main recommendations of the Health Council of the Netherlands and the Wheel is to eat less animal-based and more plant-based products (35). In this study, several limitations are set. From a health perspective, the consumption of red and processed meat is limited to a maximum of 300 g/week; from a sustainability perspective, the consumption of total meat is limited to a maximum of 500 g/week, below the current consumption. Besides limitations in product groups, there are limitations set for health reasons on SFA, TFA, and salt intake. A food product that is included in the Wheel needs to adhere to certain criteria, which are different for each of the categories of the Wheel. The FBDG has placed those products that are not included in the Wheel of Five into two categories: Daily choices and Weekly choices (**Figure 1**). The basic principle, when drawing up criteria for Daily choices, is that it should be possible to select foods from outside the Wheel of Five several times a day (33). Food products that fall outside of the Wheel and contain ≤75 kcal, ≤1.7 g SFA, and ≤200 mg Na (0.5 g salt) per portion are Daily choices. Otherwise, they are called Weekly choices. For adults, it is advised to limit the consumption of Daily choices to no more than three to five times a day, while the consumption of Weekly choices should be limited to no more than three times per week, limiting average energy intake from foods outside the Wheel to no more than 15% of total energy intake (21, 36). These criteria result in the major part of the current meat, dairy, and cheese consumption not being included in the Wheel.

Building the Model

Connecting Consumed Meat Products to Primal Meat Cuts

Judge et al. (37) was used to determine the primal cuts of beef cattle (similar data for dairy cattle were unavailable) and pork. Judge et al. (37) used data from a pre-existing data source from the Irish Cattle Breeding Federation, describing Irish beef cattle. These data were considered applicable to the Dutch beef cattle system, as many luxury beef products (i.e., prime cuts, unprocessed beef) in Dutch supermarkets originate from Irish beef. Moreover, many of the breeds mentioned in the study are known to be used as Dutch beef cattle: Charolais, Belgian Blue, and Limousin (37). Websites of Dutch butchers and chefs were used to fill the information gap (i.e., which beef and pork products originate from which primal cuts). Pork Checkoff (38) was used to determine the primal cuts of pork. The cuts of chickens were received from the experts at Plukon Food Group and Kipster.

Connecting Meat Consumption to Animal By-Products: Consumable Organs

Animal by-products are products that livestock produce that are not intended or used for human consumption in the Netherlands. Intended in this sense does not necessarily imply that the by-products are not suitable for human consumption. For instance, some organs are suitable for human consumption (e.g., not harmful to human health when consumed, for example liver and kidney); however, there is no market for these organs in the Netherlands. Other by-products are not suitable for human consumption and are instead used as feed, pet food, or biochemicals, or burned because of harmful contents (32). The cuts of carcasses of the six selected livestock animals are categorised in five components in the model: Wheel (meat,

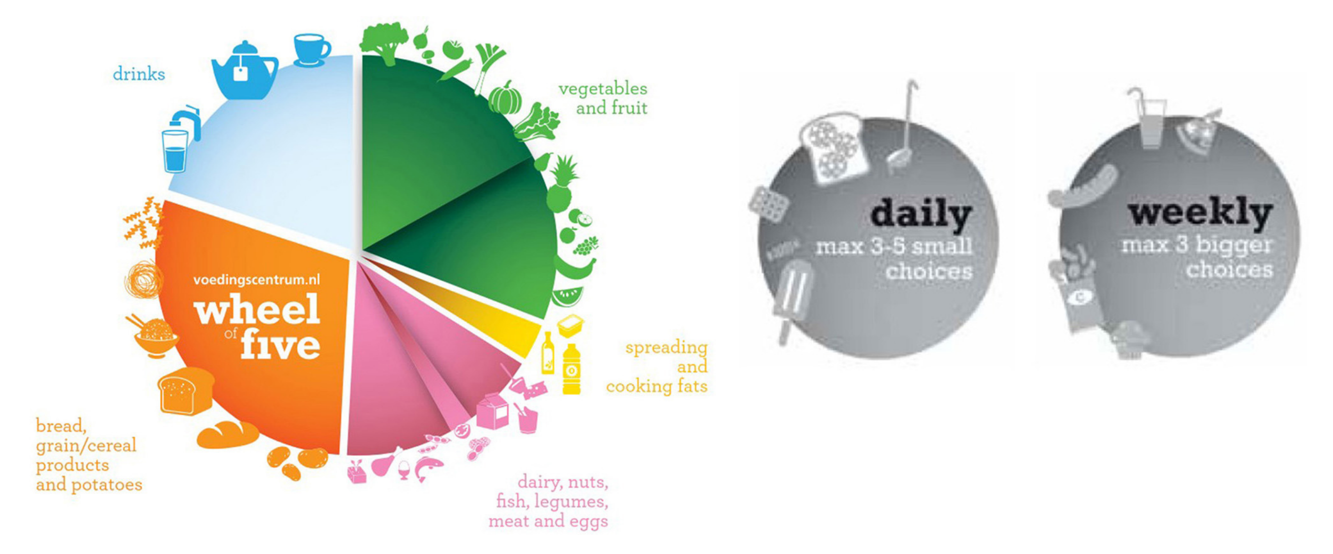

FIGURE 1 | Wheel of Five with the five food groups and a visualisation of Daily and Weekly choices (33).

TABLE 1 | Percentage of components within each livestock production system based on weight.

Livestock	Hot carcass weight (kg)	Edible weight* (kg)	Wheel (meat, excluding organs) (%)	Organs in the Wheel (liver, kidney) (%)	*Wheel, total (%)*	Daily choices (%)	Weekly choice (%)	*Non-wheel (known) (%)*	Unknown (i.e., inedible/not marketed, including bones) (%)
Dairy cattle	307	151	38	1	39	6	25	31	29
Calves	160	79	44	15	59	00	25	25	15
Beef cattle	464	212	38	1	39	6	25	31	29
Laying hens	2.15	1.35	0	0	0	0	63	63	37
Roosters	0.90	0.80	48	0	48	0	41	41	11
Broilers	1.65	1.03	44	8	52	1	9	11	38
Pigs	110	77	22	1	23	17	29	46	31

Numbers are depicted in % of edible weight unless indicated differently.
**Edible weight: excludes head, skin, and internal organs. Includes bones, fat, and moisture.*

excluding organs), organs in the Wheel, Daily choices, Weekly choices, and unknown (inedible/not marketed) (**Table 1**).

Connecting Dairy Consumption to Dairy Herd Meat and Dairy Fats Consumption

The dairy cow system is more complicated than the other livestock systems (**Figure 2**); therefore, some assumptions were made. A Dutch dairy cow delivers, on average 4.75 calves in her lifespan, so the ratio dairy cow:calf is 1:4.75. It was assumed that half of the calves will be available for their meat (i.e., the male ones, ratio 1:2.375), and that the other female half will be fully used for replacement of the dairy herd. In fact, this percentage is somewhat higher, as an unknown minor part of the female calves is also available for their meat (39). Based on slaughter weight, the model calculates the quantity of edible meat per animal. **Figure 2** shows an example of the quantities related to the recommended portions of semi-skimmed milk and low-fat cheese per year for adults 19–50 years old: 109.5 L milk and 14.6 kg cheese.

Dutch milk production per cow is calculated as on average 30,000 L per life span (39). A litre of semi-skimmed milk (1.5% fat) has a by-product of 33.8 g of butter, because cow milk has an average fat percentage of 4.1%. Per 1 kg of cheese, about 10 L of milk is needed on average. One kg of low-fat cheese (30% fat in dry weight) requires 10.69 L of milk and yields a by-product of 530.9 g of butter and 9.16 L of whey [based on (26)]. For the example in **Figure 2**, this means 3.7 kg butter from milk and 7.75 kg butter from cheese production per year, derived from 269.3 L of milk from the cow. The amount of whey produced as a by-product of cheese was depicted in the model to give an indication of its volume. Fat as by-product from dairy and cheese was modelled as butter. Fat could be processed into cream as well, which would yield a different amount of product. Whey is applied for human consumption as an ingredient for soft drinks

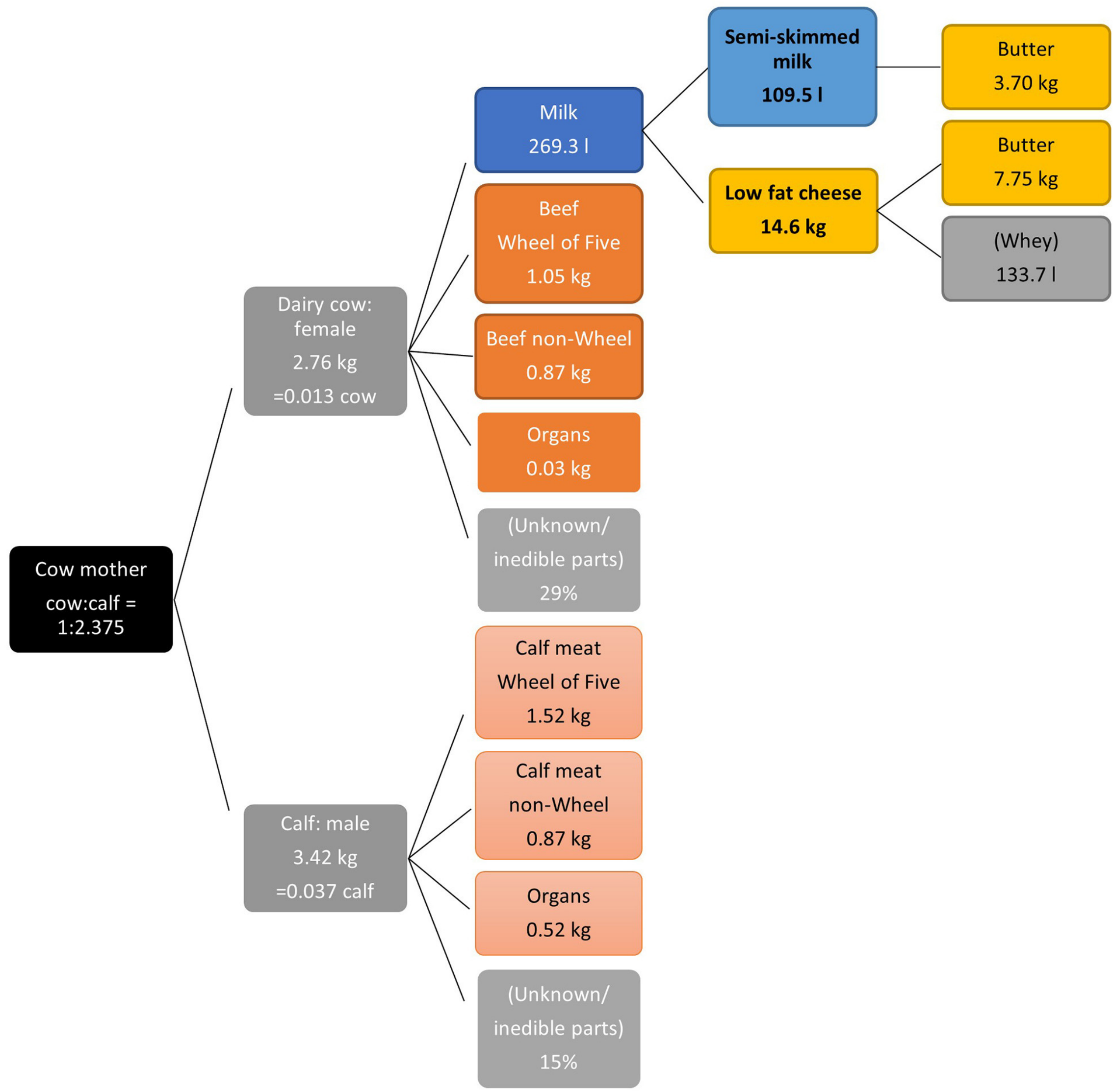

FIGURE 2 | Model with interdependencies in the dairy cow system (products in parenthesis are outside the model), illustrated by the quantities related to the recommended portions of semi-skimmed milk and low-fat cheese per year for adults 19–50 years old.

(Weekly choice) or as baby formula. However, further analysis on whey was beyond the scope of this study. Based on the ratio of 30,000 l milk:dairy cow, this amount of milk is equivalent to 2.76 kg of cow. Based on the ratio cow:calf, 2.76 kg per cow (= 0.013 cow) is equivalent to 3.42 kg calf (= 0.037 calf). The 2.76 kg beef and 3.42 kg of calf are divided in several edible and unknown/inedible parts based on the distribution given in **Table 1**.

Connecting Egg Consumption to Hen and Rooster Consumption

Egg production is an agricultural sector separate from chicken meat production. Within the egg production sector, hens are slaughtered after productive life and marketed most of the time in their entirety as chicken for soup (Weekly choice). The assumption is that in the system for every hen there is one rooster, but with a lower average slaughter weight: 0.9 vs. 2.15 kg. The lifetime egg production per chicken is on average 383 eggs (29). Per 100 g egg (two pieces of 50 g), the by-product is 7.03 g of hen meat and 4.17 g of edible rooster meat. Roosters are normally killed after birth and used as feed for pets or zoo animals. An exception is a commercial farmer in The Netherlands that raises roosters to be sold as chicken meat (www.kipster.nl). The assumption is that this business model can be upscaled to the whole sector.

Connecting Pork Consumption to Feeding Pork Solely on Residue Streams

Ruminants such as cows can be solely fed on grass, but not monogastric animals such as pork. According to Elferink et al.

(17), the Dutch food industry has four main residue streams that are used as pork feed. The potato industry has a residue stream of peals (23%), which has a quantity of 7 kg per capita per year. The sugar industry has two residue streams from sugar beets: dried beet pulp (24%) and molasses (17%), accounting for 18 and 12 kg per capita per year. Based on these inputs, an amount of pork can be raised equal to 28 g per capita per day (196 g/week). The fourth residue stream is soybean scraps, but we interpret this as a regular input stream rather than a residue stream, because it involves 80% of the soybean. This stream accounts for 72 kg of pork per capita per year, which is equal to 53 g/day (17). Chicken can also be fed on residue streams, but we have no access to data calculating the conversion form residues to chicken meat.

Connecting Food System Production to Recommended Quantities of Animal Products in Dutch FBDG

For classification of the components of the livestock production systems into Wheel product, Daily choice, or Weekly choice, the criteria from Brink et al. (21) were used (see Section Nutritional Classification of Animal Products.). Males (19–30 years old) are used as reference, because it is the population group with the highest overall consumption, consumption of meat, and environmental impact of their diet (10). **Table 2** shows the current consumption of this group per week, compared with the recommended quantities.

Regarding animal meat, it is advised to limit the consumption to a maximum of 500 g a week, of which a maximum of 300 g for red meat. The advice for dairy (e.g., semi-skimmed milk, yoghurt) is to consume two to three portions (150 mL) per day; for low fat cheese, there is a recommendation of 40 g per day. Furthermore, it is advised to consume two to three eggs à 50 g per week. Finally, the advice for spreadable and cooking fats is 65 g per day for males in the age of 19–30 years old (36). The model allows a maximum of two Daily choices per day (14 per week; = <1,050 kcal) and 1 Weekly choice per week for animal-derived products (set as a portion of 100 g meat) (allowing room for other choices such as alcoholic drinks, soft drinks, snacks, cakes, and sweets).

Model Scenarios

The model makes it possible to run a range of scenarios, but for this study seven possible scenarios were selected to evaluate the consequences of connecting recommended intakes (FBDG) to consuming all interrelated animal products: first, two scenarios to explore the impacts of the maximum nutritional recommendations (1a, 1b); second, two scenarios to explore the minimum nutritional recommendations (2a, 2b); third, two scenarios to explore the maximal utilisation of existing waste streams (3) and existing livestock (4). The scenarios are:

- Scenario 1a: current FBDG with maximum of 500 g meat, of which 300 grammes is for red meat in beef (maximum impact scenario)
- Scenario 1b as 1a: 300 g red meat of which 188 grammes is of pork and 112 g beef (realistic scenario)
- Scenario 2a: FBDG only including chicken from laying hens and beef from dairy cattle and calves (minimum impact, near-vegetarian scenario)
- Scenario 2b: FBDG as 2a near vegetarian, including chicken roosters (minimum impact, rooster scenario)
- Scenario 3: FBDG as 2b including pork raised on residue streams of the food industry (28 g pork per day) and including current beef cattle, but only grass-fed or grazing in nature sites (food system optimal scenario)
- Scenario 4: FBDG as 3 with maximal utilisation of existing Dutch livestock (broilers, beef cattle) up to 500 g meat (agriculturally optimal scenario).

The quantities of meat mentioned in scenarios 1a, 1b, 3, and 4 are the input quantities for the model. Per scenario, the environmental impact on intake of energy and five nutrients, limits in consumption related to the maximum of 2 Daily and 1 Weekly choices, and effects on the livestock size were evaluated (assuming the whole population eats according to the scenario). Based on the results of Scenarios 1-4, Scenario 5 (nutritionally optimal scenario) was calculated at the end, looking at maximal utilisation of livestock within the nutritional recommendations, i.e., keeping the Daily and Weekly choices within the limits of FBDG and including all livestock systems in a more optimal amount (300 g white and a maximum of 200 g red meat).

RESULTS

Consumption of Animal Products per Scenario

Table 3 gives an overview of the consumption of animal products per scenario in grammes per week. The inputs for the model were the quantities of eggs, milk, and cheese, set as constraints for the scenario. The outputs of the model were quantities of animal meat (laying hens, veal, dairy cattle, etc.).

Part of Animal Consumption Within the Wheel of Five

Table 1 demonstrates that different animals have a different yield in total Wheel products, ranging from 0 (laying hens consumed in whole such as in soup) to 52% for broilers and 59% for calves of the hot carcass weight (i.e., the carcass excluding the head, skin, and internal organs and including bones, fat, and moisture). This is related to the percentage of available lean and fresh, unprocessed cuts. Based on the available data, organ consumption has high potential for broilers (13%) and beef (calves: 15%). Broilers and calves fit best into the Wheel, and laying hens do not fit at all, because they are sold as one piece. Pigs are the livestock with the lowest part lean, unsalted, unprocessed meat of 22%. This means that in practise of a food system approach, which includes eating the animal from nose to tail, along with every portion of beef in the Wheel, a portion of beef Weekly choice is expected to be eaten. For pork, along with every portion in the Wheel, two portions of pork Weekly choice are expected to be consumed, and for broilers, along with every portion in the Wheel, 0.5 portions of broiler Weekly choice.

TABLE 2 | Recommended intake of animal products (g/mL) for adults per week in The Netherlands (36) and the current consumption of males 19–30 years old (27) in g/week.

	Minimum recommendation (g/week)	Maximum recommendation (g/week)	Current consumption (g/week) (males 19–30 y)
Total meat	0	500	864
Red meat (beef, pork)	0	300	683
White meat (chicken)	0	500 minus red meat	181
Milk products (milk, yoghurt)	2,100	3,150	2,389
Cheese (low fat 40 g/day)[a]	210	280	258
Butter (preference: soft liquid oils)[b]	0	84 semi-skimmed	8.2
Spreadable and cooking fats	455	–	165
Eggs (2–3)	100	150	85
Fish (1 portion)*	100	–	109

[a]For mathematical reasons, a lower limit for cheese was required for the model; thus, the lower limit for cheese was set at 30 g a week, which is in line with previous Dutch FBDG.
[b]1 portion butter is 6 g, 44 kcal, 3.2 g saturated fat and < 0.1 g salt (Weekly choice), semi-skimmed butter is 20 kcal, 1.5 g saturated fat, 24 portions butter = 1,050 kcal, 143 g/week.
**Fish is not included in this study.*

TABLE 3 | Consumption of animal products per scenario in g/week.

Scenario	Eggs	Dairy		White meat			Red meat					Total meat
		Cheese	Milk	Broilers	Roosters	Laying hens	Pork	Beef cattle	Veal	Dairy cattle	Total red meat	
1a	150	280	3,150	180	0	11	0	191	66	44	300	491
1b	150	280	3,150	189	0	11	188	0	67	45	300	500
2a	100	210	2,100	0	0	7	0	0	46	31	77	84
2b	100	210	2,100	0	4	7	0	0	46	31	77	89
3	100	210	2,100	0	4	7	196	17	48	32	292	304
4	150	280	3,150	183	6	11	171	17	67	45	300	500
5	150	125	2,100	283	6	11	40	17	38	25	120	420

TABLE 4 | Nutritional intake per day by consumption of animal (-derived) products of differing scenarios and of reference (=current) diet.

Scenario	Energy (kcal/day)	Animal protein (g/day)	SFA (g/day)	TFA (g/day)	Salt (g/day)
Reference values (males 19–30 y)		36.4 (40%)	29.5 (10 en%)	3.0 (1 en%)	6.00 (2.17 from animal products)
Reference diet	885	*58.6*	23.7	0.66	*2.82*
1a	712	*44.8*	*30.3*	0.76	1.55
1b	733	*44.5*	*31.4*	0.72	1.84
2a	444	23.7	20.5	0.49	0.94
2b	444	23.8	20.5	0.49	0.94
3	511	29.6	22.6	0.52	1.39
4	723	*44.6*	*31.4*	0.73	1.81
5	451	30.8	17.5	0.40	1.00

In italics the exceeded intakes compared to the reference values. Reference values for males 19–30 years old are based on a 2,645-kcal diet, 36% salt intake from meat and dairy, and reduction of animal-based protein intake to 40% (27, 36).

Effect on Intake of Saturated Fats and Salt

Reduction in the consumption of meat will result in changes in nutrient intake. Considering not only recommended meat cuts in the model but also Daily and Weekly choices result in an increased consumption of saturated fatty acids (SFAs), trans-fatty acids (TFAs), and salt. Moreover, compared with the current diet, the daily intake of SFA and TFA is higher in all maximum scenarios (i.e., upper limits of FBDG, see **Table 4**), but lower in all minimum scenarios (i.e., lower limits of FBDG, scenarios 2a, 2b, and 3). Only scenarios 1a, 1b, and 4 exceed the current consumption of SFA and TFA in the reference diet and the reference value for SFAs. These scenarios also contain more than 40% protein from animal sources. Salt intake decreases in all the scenarios and is below the reference value of 2.17 g. Scenario 5 demonstrates the best results for SFAs, TFAs, and salt (and calories) and still provides a substantial portion of animal protein (30.8 g/day).

Additionally, the consumption of dairy and eggs is inevitably linked to some beef meat (dairy cow and calf) and chicken meat (laying hen and rooster). More specifically, more beef meat is available than chicken meat regardless of the (lower or upper) limits of the FBDG. Consumption of dairy and eggs following scenario 2a, in case no extra streams of meat exist (no beef cattle nor broilers) and in case no roosters are consumed (like in the current situation), 77 g of beef meat per week (ca. 1 portion of 100 g each 9 days) vs. 7 g of chicken meat per week (ca. 1 portion each 14 weeks) is available. Thus, when following the FBDG on dairy and eggs in the current situation, consumption of about 1 portion of beef (dairy cow and calf) each 6 days is still within the borders of the food system, whereas the consumption of chicken (laying hen) is much less frequently allowed by the food system: one portion each 9 weeks (scenario 2b).

Results on GHGE and LU

Table 5 summarises the GHGEs and LU per scenario, compared with the planetary boundaries. According to Wood et al., the boundaries for food per person are respectively, 0.89 kg CO_2-eq/day and 2.68 m^2*year/day (4). Concerning GHGE, consumption of animal food products according to the FBDG in the current situation (i.e., in a food system without roosters and little veal but with beef cattle and broilers) leads to GHGE of 3.34 kg CO_2-eq/day (Scenario 1a). This diet, following the upper limits of FBDG, still leads to lower GHGE than the reference diet (3.53 kg CO_2-eq/day), but also exceeds the planetary GHGE boundary per day. Scenario 2a (minimum impact, near-vegetarian scenario) demonstrates the lowest climate impact (1.46 kg, −59%) but still exceeds the planetary boundary. Regarding LU, consumption of animal food products according to the FBDG in the maximum impact scenario leads to an LU of 2.44 m^2*year/day (scenario 1a), which is more than the LU because of the current diet (2.03 m^2*year/day), but safely within the planetary LU boundary of 2.68 m^2*year/day (**Table 5**). In fact, LU for animal food products stays within the planetary boundary in all the scenarios.

The least greenhouse gas emissions (GHGEs) and land use (LU) within the Food-Based Dietary Guidelines (FBDG) can be achieved in a food system scenario without extra streams of meat (i.e., no beef cattle or broilers) and without roosters (Scenario 2a; minimum impact near-vegetarian scenario). Scenario 2b (minimum impact, white meat scenario) is nearly the same.

Limiting Factors in Meat and Dairy Consumption

The results show that in all scenarios the category of Daily choice is a limiting factor, i.e., the caloric intake due to Daily choices exceeds 1,050 kcal/week (**Table 5**). The main contributor to the caloric intake of Daily choice is dairy fat (in the model as butter). By advising semi-skimmed milk, the dairy cow system produces for every daily portion of 450 ml of semi-skimmed milk (1.5% fat) 15.2 g of butter, and for every daily portion of 40 g of low-fat cheese (30% fat in dry matter; 18% fat) 21.2 g of butter. In total, 36.4 g of butter is more than six daily portions (Daily choice). The current situation is that most of this butter is exported, but if butter is to be consumed within the food system, it will be the food product that causes imbalance and excess kcal and saturated fat intake, and will be limited in the model. Indeed, further analysis excluding the caloric contribution of butter to Daily choice showed that Daily choice was far below the maximum recommendation of 1,050 kcal/week (**Table 5**; column 4 in parenthesis).

Besides the category of Daily choice, the category of Weekly choice is limiting in four of the scenarios, i.e., the amount of animal (-derived) products categorised as Weekly choice exceeds 100 g/week. The reason for exceeding the Weekly choice is primarily due to the consumption of pork, the most consumed meat, but the consumption of beef is also a contributor.

In contrast, the category of Weekly choice is not limiting in the following scenarios:

- in a situation in which no extra streams of meat exist (no beef cattle nor broilers) and no roosters are consumed (Scenario 2a minimum impact, near-vegetarian),
- in a situation in which no extra streams of meat exist but roosters are consumed (scenario 2b minimum impact, rooster meat), and
- in Scenario 5 (nutritionally optimal) in which Weekly choice is limited to 100 g/week.

Livestock Size Impact

Dutch adults consume an average of 98 grammes of meat and meat products per day. Men eat more meat and meat products (115 g/day = 805 g/week) than women [81 g/day = 567 g/week; (27)]. Dutch adults prefer pork (47%), followed by chicken (29%), and beef (20%). Consumption of calf is very low: 1.7% (40). As the consumption of other animal species is <3%, it is excluded from this study. The Dutch self-sufficiency rates show that production and consumption in the country do not align (41). Especially, the veal industry produces substantially more than the Dutch population consumes, leading to a self-sufficiency rate for veal of 734%. All animal products have a self-sufficiency rate >100% (poultry 191%, eggs 314%, cheese 222%, butter 153%) except for (non-dairy herd) beef meat (59%). National beef cattle provide, on average, 17 g of beef *per capita* per week (25).

In all the scenarios, the recommended amounts of eggs, dairy, and cheese could be provided by a livestock size smaller than the current one, except for beef cattle in Scenario 1a (**Table 6**). Compared with current meat production numbers, consumption of animal (-derived) food products according to the FBDG in the current situation (i.e., in a food system without roosters but with beef cattle and broilers) requires annually just 51% of the dairy cows; 47% of the calves; 26% of the laying hens; and 26% of the broilers (Scenario 1a). In the food system optimal scenario (3), only a third of the cattle, a quarter of the laying hens, and a sixth of the pigs are needed. In the agriculturally optimal scenario (4), the livestock could be halved (except for beef cattle) to fulfil the FBDG-recommended amounts for the total population. In all the scenarios, a maximum of 14% of the current pig livestock is consumed.

TABLE 5 | Overview of maximum nutrient intake recommendations exceeded (in italic) in the different scenarios.

Scenario	Which boundary exceeded?				
	GHGE [kg CO_2-eq/day]	LU [m^2*year/day]	Daily choice [kcal/wk] (without butter)	Weekly choice [g/wk]	Explanation (main contributor(s) between brackets)
FBDG boundary (36)			1,050	100	
Planetary boundary (4)	0.89	2.68			
Reference diet (10)	*3.53*	2.03			
1a maximum impact	*3.34*	2.44	*1,846* (42)	*142*	Daily (butter, beef cattle, broilers); Weekly (beef cattle, broilers).
1b realistic	*2.73*	1.62	*1,970* (90)	*154*	Daily (butter, pork, broilers); Weekly (pork, broilers, beef cattle).
2a minimum impact, near-vegetarian	***1.46***	**0.80**	*1,292* (4)	33	Daily (butter).
2b minimum impact, rooster meat	*1.47*	0.81	*1,292* (4)	35	Daily (butter).
3 food system optimal	*1.86*	1.15	*1,428* (84)	*122*	Daily (butter, pork, beef cattle); Weekly (pork, beef cattle, roosters).
4 agriculturally optimal	*2.79*	1.69	*1,965* (86)	*155*	Daily (butter, pork, broilers, beef cattle); Weekly (pork, beef cattle, broilers).
5 nutritionally optimal	*1.93*	1.20	1,048 (37)	100	Daily (butter, pork, broilers).

Ecological impact per day (of the animal product consumption according to current Dutch FBDG) of the different scenarios vs. the current diet and planetary boundaries. Figures in bold are the lowest (4, 10).

TABLE 6 | Estimated amounts of animals in the livestock production systems needed per year in the different scenarios, extrapolated to the Dutch population.

Scenario	Dairy cattle	Calves	Beef cattle	Laying hens	Roosters	Broilers	Pigs
1a	51%	47%	1,121%	39%	–	26%	–
1b	51%	47%	–	39%	–	27%	14%
2a	36%	33%	–	26%	–	–	–
2b	36%	33%	–	26%	29%	–	–
3	36%	33%	100%	26%	29%	–	14%
4	51%	47%	100%	39%	44%	26%	13%
5	29%	26%	100%	39%	44%	40%	3%
Production (100%)	522,300	1,629,800	71,500	17,951,700	= hens	605,487,800	15,907,000

A hyphen means exclusion from the model in that specific scenario. Bottom row depicts current number of animals slaughtered for meat production in 2018 = 100% (25).

DISCUSSION

The Food System Approach

This study explored the interconnection between food consumption and production in different food system scenarios. The main finding is that when taking interdependencies within the animal food system/livestock systems into account, it will affect the recommended intake of animal-based foods. Applying a more sustainable food system approach (i.e., eating the whole animal, utilising animal by-products, using waste streams) results in a decrease in GHGE, LU, and number of livestock compared with the current situation, but in some scenarios, it also increases the intake of SFAs, TFAs, and salt, and in most scenarios, it exceeded the recommended amount of Daily and/or Weekly choices. This approach asks for reconsideration of quantities of recommended cheese, total meat, and red meat, and for adaptations in consumer preferences for non-popular cuts, organ meat, veal, hens, roosters, and saturated fat source. The conceptual framework of this study may be useful in the discussion on how future sustainable FBDG can incorporate a more food system-based approach.

The main strength of this study is its novelty. Earlier research studied potential dietary changes, such as better adherence to healthy dietary patterns that could reduce the ecological impact of the diet, but not within a food system approach. For example, Vellinga et al. (10) evaluated the ecological impact of Dutch food consumption patterns by regression analysis and found that better adherence to Dutch healthy diet guidelines (35) for red and processed meat (less consumption) and vegetables (more consumption) was most strongly negatively associated with GHGE. This is in line with the findings of Van Dooren et al. (42) showing that eating a recommended healthy diet in compliance with the Dutch FBDG (2006) or other healthy diets such as the New Nordic and Mediterranean diet is likely to result in less GHGE and LU (42). This study shows for the first time that part of these conclusions (i.e., on red and processed meat) indeed also applies if the interconnections within the food system are considered.

Because of a lack of data, several assumptions were made, and several minor side streams were excluded, such as separator meats, unpopular organs such as intestines or hearts, and animal fat tissue. One of the arguable assumptions is that the ecological impact of all beef meat products is the same (beef and dairy cattle). Nevertheless, ecological impact data of beef and dairy cattle are only available on the level of the whole animal, and the differences are not to be neglected: the GHGEs and LU of beef cows are roughly four and five times higher than those of dairy cows, respectively (29). Also, only the highly productive Dutch livestock system was taken into consideration. Moreover, only few nutritional aspects were taken into consideration, without possible consequences for vitamin and mineral intake. Therefore, this study is more relevant for the conceptual framework including a novel food system approach rather than for its exact outcomes.

Nevertheless, several steps need to be taken to transform the current Dutch livestock production system and consumption of animal (-derived) products for all of the scenarios, even for the agriculture optimal one (4). In the end, many factors besides nutrition, including but not limited to politics, economics, health, ecology, and ethics together will shape the future food system. This study demonstrates only the impacts of possible scenarios from a public health and ecological food system approach.

Economic Allocation of Ecological Impact

One of the most important and notable aspects in sustainable food research in general is that the current use of economic allocation is crucial, as it has an enormous effect on the ecological impact figures. Especially for by-products that have low ecological impacts according to this approach, i.e., roosters, laying hens, calves, animal fat, due to which the consumption of these products would theoretically be favourable from an environmental point of view (as in Scenario 3). Also, from a financial point of view, these by-products have the preference, as they come from waste or rest streams (i.e., pet food and feed) that currently are not used for human consumption. Such by-products, which are "free of charge" from an ecological impact perspective, disappear from the current, local human food system (i.e., these by-products end up in pet food and feed, or are exported). Meanwhile, part of the ecological impact has been made already in the country of origin. Therefore, from this perspective, using products, such as palm oil from abroad rather than local butter or animal fats, seems to be odd. This example demonstrates the ecological preference for animal by-products over additional imported ingredients with additional land use. Alternatives for economic allocation of ecological impact are physical allocation approaches such as mass or energy allocation (43). However, results in terms of GHGE and LU will inevitably be highly dependent on the choice of allocation (29, 43).

In the minimum scenario (2a, 2b), the amount of beef meat (dairy cow and calf) available in a diet is much more than the amount of chicken (laying hen) available. Contrary to this result, existing ecological impact data show that 1 kg of beef meat is less sustainable than 1 kg of chicken meat (28, 30). This contradiction can be explained by the fact that this study with a food system approach made use of existing ecological impact data that were not generated from a food system perspective.

Considerations From the Nutrition Perspective

The focus of this study was to identify food products that would reduce the ecological impact without affecting health benefits substantially, to make optimal use of the interlinkage between animal (-derived) products between production and consumption. However, the health perspective should not be forgotten, as some by-products might be less healthier than the products it may substitute [for instance the high content of SFAs in butter vs. those in olive oil or sunflower oil (23)]. The results show that in some cases, using these by-products from rest streams with a minimum ecological impact does fit in a healthy dietary pattern, for instance organ and rooster meat that are included in the Wheel.

Another way to make optimal use of the interlinkages in the system of animal (-derived) products between production and consumption is to consume all safe parts of an animal rather than just the Wheel products. The results show that roughly a third (pork) to half (beef and chicken) of the hot carcass weight is classified as Wheel products. Consuming only the Wheel parts entails wasting a substantial part of the animal that is safe for human consumption. What is more, consumption of only Wheel parts requires a larger number of animals for the same amount of (Wheel) meat. In contrast, if we would consume all safe parts of the animals, food waste as well as the livestock size could be minimised (without taking export into account). An example are parts of pork that are nowadays very low in consumption (i.e., black pudding, cheeks, pork belly), most of which could be labelled Weekly choice. The drawback of such advice for the consumer is that the freedom to choose outside the Wheel will be limited.

Keeping the health perspective in mind, aligning production and consumption of animal products can also be stimulated when only Wheel products are taken into consideration (e.g., chicken fillet and chicken leg). However, the consumption of such Wheel products is not in line with the natural ratio of these components. According to a major chicken slaughterhouse, chicken fillet and chicken legs (i.e., drumsticks and chicken thighs) comprise about 24 and 48%, respectively, of the animal, giving a natural ratio fillet:legs of 1:2, whereas the current consumption of these parts is, respectively, 17.7 and 17.9 g/day, resulting in a "consumption ratio" fillet:legs of about 1:1. Consequently, in order to eat within the borders of the food system, either the consumption of chicken fillet should decrease by half or the consumption of chicken leg should double.

Based on the results, we calculated an extra Scenario 5 to keep the Daily and Weekly choices within the limits of the FBDG. This calculation demonstrates that cheese consumption and recommendation should be limited to about 20 g/day (145 g/week) and milk to the current lower recommendation of 300 mL/day. The maximum space for meat consumption in this scenario is 410 g of which a maximum of 140 g is for red meat. Both adjustments would also result in reduction of the environmental impact (GHGE −45%, LU −41%). However, we did not analyse the effects of these reductions on micronutrient intake yet, which is needed to check for sufficient intakes of, e.g., calcium, iron, and B-vitamins for Scenarios 2a, 2b, and 3. We recommend this additional analysis for future studies. The other scenarios fit completely within the guidelines. For nutrients that are mainly supplied by meat in the Dutch diet, the dietary reference level is not always achieved, both for Dutch vegetarian diets and for diets including meat. These are small differences with the standard, where the scenarios provide a level that is above or comparable with the current consumption (36). This is already taken into account through specific recommendations for meat-free diets within FBDG, e.g., it is recommended to consume foods naturally rich in iron, to use sufficient dairy and wholegrain products, and to consume meat replacements with sufficient protein and enriched with iron and thiamine or vitamin B12 (21). This is less urgent for the scenarios with a meat consumption of 84 to 500 g per week, although adequate replacement is recommended for all consumers.

Scenarios with a higher white meat consumption (1b, 4) demonstrate its positive effects on nutritional intake (SFAs, TFAs, salt) and GHGE, whereas scenarios with higher red meat consumption, such as high in pork (1b, 3, 4), demonstrate a negative effect on nutritional intake. Therefore, Scenario 5 (high white, low red meat) shows the best compromise for both health and sustainability, based on the indicators used in this study.

Considerations From the Ecology Perspective

The results show that the Dutch FBDG regarding animal (-derived) products in the current situation can make steps forward to contribute to a more sustainable food system. The current GHGEs of the diet exceed the planetary boundary even if consumption is according to the lower limits of the FBDG. This is mainly because of beef cattle and dairy (especially cheese). In contrast, the FBDG do not exceed the planetary LU boundary. An explanation for this is that Dutch livestock production systems are relatively efficient (i.e., high milk and egg production per square metre) compared with those in other parts of the world, leading to an LU lower than the global limit. Moreover, GHGE exceeding the global boundary can be explained by the relatively large size of the Dutch animal husbandry (intensive use of inputs, fossil fuels, and infrastructure) and its inevitable emissions. Possible solutions are systems based on residue streams (17) or extensive farming of beef [grass-fed systems (44)].

Although the planetary LU boundary was not exceeded, the LU in Scenario 1a was calculated to be higher than the LU by the current reference diet. This might be explained by two assumptions of the model. First, the model assumed that butter was the only fats and oils product consumed in the FBDG, whereas the current Dutch population, in fact, consumes a mixture of animal and plant-based fats and oils, of which palm oil, for instance, has a lower LU than butter (32). Second, the model assumed a higher consumption of veal than currently consumed in the Netherlands, since most veal is exported (25). Existing data show that veal has a higher LU than dairy cows (28, 29). This study shows an evident gap between the production and consumption of veal. The previously mentioned 300 g of red meat available in Scenarios 1a, 1b, and 3 consists of almost a quarter of calf meat (67 g/week). In contrast, the current consumption of calves is only a tenth of this [1.7% of total meat (40)].

The current livestock size for meat production is substantially larger than necessary for a dietary pattern following the FBDG within a more optimal food system (Scenario 3): ca. three (calves and laying hens), to four (broilers), to six times (pigs) larger. This is in line with Dutch consumption data showing that the Dutch population consumes more meat in general than is advised by the FBDG (45). It is also in line with the fact that the Dutch agriculture is not focused on circularity, but on export (26). Interestingly, the results also show that the size of beef cattle would need to be five to six times larger than the current size in order to provide a diet with maximum of red meat as beef (Scenario 1a) within the FBDG. These extra beef cattle are not necessary in a scenario with more pork (1b). However, both scenarios underutilize the quantity of dairy cow beef available. The current dairy cow herd has this size, because there is a large export of cheese and other dairy products. Even in the agriculturally optimal Scenario 4, there is a significant reduction in livestock size for the internal market. The high rate of self-sufficiency at this moment (i.e., export dependency) is essential for the income of farmers. The stepwise transition to a more circular system with lower emissions, therefore, creates challenges to find other models of farming or sources of income.

Most of the non-meat portion of an animal carcass is biologically nutritious and can be made microbiologically safe for human consumption. However, because of individual preferences, the popularity of consumption of these products has decreased in contrast to the fact that meat consumption has increased (46). Edible by-products such as organs were widely used in culinary tradition in Europe, South America, North America, Asia, Africa, and Australia. In Africa, all parts of animals are still processed and commonly consumed as food for humans. A more efficient utilisation of animal by-products in the Netherlands and other western countries can alleviate the prevailing cost and scarcity of feed materials, which have high competition between animals and humans (47). This will also aid in reducing environmental pollution and increase the ecological efficiency of an animal-based food system.

The described dietary scenarios are more or less theoretical, including utilising old laying hens and non-popular parts of old dairy cows. The willingness of consumers to buy and pay for less than premium meat products remains to be seen.

Limiting Factors in the Scenarios

Looking at the results in detail, we see three limiting factors in the scenarios: butter, beef cattle, and saturated fatty acids (SFAs).

- In all the scenarios, butter is the limiting factor by being the main contributor to the high caloric intake in the Daily choice category. Further analysis showed that lowering the recommendation of cheese from 40 to 20 g/day solved this problem. In the model, butter was chosen to be a Daily choice, because its caloric intake per portion of 6 g is only about half of the maximum caloric intake of one Daily choice (44 vs. 75 kcal), even though it does not fulfil the criteria for SFAs. Further research could, for example, investigate whether butter can replace other cooking fats and spreads that are based on plant-based fats with high SFA content and high environmental impact, i.e., palm fat and coconut fat (48).
- Pigs and beef cattle livestock systems are the main limiting factors concerning Weekly choice. From a food system perspective, dairy cattle and calves are given priority above beef cattle because of dairy production. Following this argumentation, beef cattle is redundant and even has a negative impact on nutritional intake through additional Weekly choices while having a diet according to the FBDG in the current situation. Whenever both beef cattle and broilers are absent, no nutritional limits (i.e., such as Weekly choice) are exceeded. Still, a limited quantity of beef cattle is possible from a food system perspective, no higher than the current production of beef cattle, and preferably from grass fed cattle, or cattle raised in nature sites (see scenario 3).
- A diet following the upper limits of the FBDG, regardless of whether extra meat streams are present, leads to a higher intake of SFAs and TFAs than the current intake from animal food products. One explanation is the substantial by-production of butter due to milk and cheese consumption, which is included in the calculation of nutritional intakes. Another explanation is the higher content of SFAs in the Weekly choices due to less lean and more processed meat. Another possibility is that the model in this study includes more room for Daily and Weekly choices, whereas the current FBDG contains amounts of Wheel products providing 85% of energy needs (36).

Possible Steps to Make Guidelines More Sustainable

To move toward more sustainable livestock production systems and a balanced consumption of animal (-derived) food products, several steps can be taken to improve the current guidelines:

1. First, introducing the concept of consuming animals from nose to tail, and keeping the consumption of Weekly choice, SFAs, and TFAs within the FBDG limits would lead to lower maximum advice of red and total meat. This concept includes the promotion and consumption of less popular (lean) cuts and organs.
2. Second, recommending dairy and eggs from a health perspective in FBDG inevitably includes recommending a minimum consumption of meat on a population basis when adopting a food system approach. These quantities are low in a high productive food system. From this food system approach, vegetarian diets are less optimal than near-vegetarian diets. Considering waste streams, there would be some room for the consumption of pork (and broilers) fed on residue streams.
3. Third, introducing food system interdependencies includes the consumption of butter besides semi-skimmed milk and cheese. The limitations in SFAs, TFAs, and number of Daily choices, includes a limitation in butter and, therefore, dairy. A possible adaptation is to lower the recommendation of cheese, but cheese is also an important source of, e.g., calcium, and micronutrient intakes were not yet analysed. However, past optimisation studies from the group of the authors demonstrated that intakes of calcium and vitamins D, A, and B12 did not compromise lower quantities of milk and exclusion of cheese (42, 49).

These recommendations are in line with the ranges for animal products given by the EAT Lancet reference diet, but the averages are somewhat higher. This is because of the application to the local context of this study and the lack of a food system approach by Willett et al. (3).

Other sustainability scenario studies also suggest that within Dutch eating habits, satisfying optimisation constraints required a shift away from beef, cheese, butter, and snacks toward plant-based foods and fish and shellfish, questioning acceptability (50). A great deal of hope has been placed lately on a flexitarian diet to help solve food-related environmental sustainability problems. There is a growing group of flexitarians and vegetarians who would likely accept the described scenarios, but this is distinct from a substantial group of consumers who are deeply attached to meat-eating and have no intention whatsoever to limit their meat intake, let alone their already changing meat-eating behaviours (51). Other steps toward a more sustainable food system approach include minimising the production and consumption of beef cattle, pork, and broilers, choosing local animal (by-)products over foreign comparable products, maximising consumption of the whole animal, and exploring alternative applications of butter instead of plant-based saturated fats. Following these steps will help to prevent waste of rooster meat, organ meat, and other animal (by-)products considerably and would lower the number of livestock needed to fulfil a balanced, healthy consumption of animal (derived) products. The advice for future research is to also look at other food system interdependencies, for example, soy oil and soy pulp, fatty fish, and bycatch, grains, and legumes in agricultural rotations.

CONCLUSIONS

This study provides a first insight into how the Dutch FBDG could be adapted to better align production and consumption of animal(-derived) food products within the Dutch food system, showing that the current FBDG could make steps toward a more sustainable food system when interdependencies in the animal production system are included. The major strengths of this study are its food system approach and its model, which can also be used to explore other food system scenarios. The main limitation is the lack of detailed LCA data generated with a food system approach. Hence, future studies on food systems need to be aware of the data gap on ecological impact data of different livestock types. Food systems-thinking involves shifts in the use of different livestock types in different livestock production systems. Addressing the data gap on differing livestock breeds is a first yet substantial subject for research to substantiate the change toward future sustainable food systems. Therefore, this study is more relevant for the conceptual framework and novel food system approach rather than for its exact outcomes. The conceptual framework of this study may be useful in the discussion on how future sustainable FBDG can incorporate a more food system-based approach, in addition to other preconditions applied to FBDG development, such as lower and upper level of nutrients, recommendations on food groups by the health council, and dietary habits of the population.

DATA AVAILABILITY STATEMENT

The Excel model with the data supporting the conclusions of this article will be made available by the authors on request, without undue reservation.

AUTHOR CONTRIBUTIONS

LM: methodology, investigation, formal analysis, and writing—original draft. CD: methodology, conceptualisation, formal analysis, writing—original draft, and visualisation. MS: writing—review & editing. SB: writing—review & editing and supervision. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. UN. *Transforming Our World: The 2030 Agenda for Sustainable Development.* New York, USA: United Nations (2015).
2. HLPE. *Nutrition and Food Systems.* Rome, Italy: Committee on World Food Security (2017).
3. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4
4. Wood A, Gordon LJ, Röös E, Karlsson J, Häyh,ä T, Bignet V, et al. *Nordic Food Systems for Improved Health and Sustainability: Baseline Assessment to Inform Transformation.* Stockholm, Sweden: Stockholm Resilience Centre (2019).
5. Tilman D, Clark M. Global diets link environmental sustainability and human health. *Nature.* (2014) 515:518–22. doi: 10.1038/nature13959
6. Steffen W, Richardson K, Rockström J, Cornell SE, Fetzer I, Bennett EM, et al. Planetary boundaries: guiding human development on a changing *planet. Science.* (2015) 347:6223. doi: 10.1126/science.1259855
7. Fanzo J, Bellows AL, Spiker ML, Thorne-Lyman AL, Bloem MW. The importance of food systems and the environment for nutrition. *Am J Clin Nutr*. (2020) 113:7–16. doi: 10.1093/ajcn/nqaa313
8. IPCC. *Climate Change and Land, Summary for Policymakers*. Geneva: IPCC (2019).
9. Vringer K, Benders R, Wilting H, Brink C, Drissen E, Nijdam D, et al. A hybrid multi-region method (HMR) for assessing the environmental impact of private consumption. *Ecol Econ*. (2010) 699:2510–6. doi: 10.1016/j.ecolecon.2010.07.027
10. Vellinga RE, van de Kamp M, Toxopeus IB, van Rossum C, de Valk E, Biesbroek S, et al. Greenhouse gas emissions and blue water use of dutch diets and its association with health. *Sustainability*. (2019) 11:6027. doi: 10.3390/su11216027
11. Van de Kamp ME, Van Dooren C, Hollander A, Geurts M, Brink EJ, van Rossum C, et al. Healthy diets with reduced environmental impact?–The greenhouse gas emissions of various diets adhering to the Dutch food based dietary guidelines. *Food Res Intern.* (2018) 104:14–24. doi: 10.1016/j.foodres.2017.06.006
12. Tetens I, Birt CA, Brink E, Bodenbach S, Bugel S, De Henauw S, et al. Food-Based Dietary Guidelines – development of a conceptual framework for future Food-Based Dietary Guidelines in Europe: report of a Federation of European Nutrition Societies Task-Force Workshop in Copenhagen, 12–13 March 2018. *Br J Nutr*. (2020) 124:1338–44. doi: 10.1017/S0007114520002469
13. FAO and WHO. *Sustainable Healthy Diets – Guiding Principles.* Rome, Italy: FAO and WHO (2019).
14. Gonzalez Fischer C, Garnett T. *Plates, Pyramids, Planet; Developments in National Healthy Sustainable Dietary Guidelines: A State of Play Assessment*. Rome, Italy: FAO (2016).
15. Health Council. *Guidelines for a Healthy Diet: The Ecological Perspective.* The Hague, Netherlands: Gezondheidsraad (2011).
16. Kovacs B, Miller L, Heller MC, Rose D. The carbon footprint of dietary guidelines around the world: a seven country modeling study. *Nutr J.* (2021) 20:15. doi: 10.1186/s12937-021-00669-6
17. Elferink EV, Nonhebel S, Moll HC. Feeding livestock food residue and the consequences for the environmental impact of meat. *J Clean Prod.* (2008) 16:1227–33. doi: 10.1016/j.jclepro.2007.06.008
18. Van Dooren C, Marinussen M, Blonk H, Aiking H, Vellinga P. Exploring dietary guidelines based on ecological and nutritional values: a comparison of six dietary patterns. *Food Policy.* (2014) 44:36–46. doi: 10.1016/j.foodpol.2013.11.002
19. Van Zanten HH, Herrero M, Van Hal O, Röös E, Muller A, Garnett T, et al. Defining a land boundary for sustainable livestock consumption. *Global Change Biol.* (2018) 24:4185–94. doi: 10.1111/gcb.14321
20. Springmann M, Spajic L, Clark MA, Poore J, Herforth A, Webb P, et al. The healthiness and sustainability of national and global food based dietary guidelines: modelling study. *BMJ.* (2020) 370:m2322. doi: 10.1136/bmj.m2322

21. Brink E, van Rossum C, Postma-Smeets A, Stafleu A, Wolvers D, van Dooren C, et al. Development of healthy and sustainable food-based dietary guidelines for the Netherlands. *Public Health Nutr.* (2019) 22:2419–35. doi: 10.1017/S1368980019001435
22. Nelson ME, Hamm MW, Hu FB, Abrams SA, Griffin TS. Alignment of healthy dietary patterns and environmental sustainability: a systematic review. *Adv Nutr.* (2016) 7:1005–25. doi: 10.3945/an.116.012567
23. Voedingscentrum. *Dutch Branded Food Database (Levensmiddelendatabank).* Voedingscentrum The Hague, Netherlands (2019). Available online at: www.levensmiddelendatabank.nl (accesed December 18, 2020).
24. CBS StatLine. *Agriculture, Crops, Animals and Land Use by Farm (In Dutch: Landbouw; gewassen, dieren, grondgebruik naar hoofdbedrijfstype, regio).* The Hague, Netherlands (2020). Available online at: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/80783ned/table?ts$=$1588060216286 (accessed March 3, 2020).
25. CBS StatLine. *Meat Production, Number of Slaughters, Carcass Weight per Animal (In Dutch: Vleesproductie; aantal slachtingen en geslacht gewicht per diersoort)*. The Hague, Netherlands (2020). Available online at: https://opendata.cbs.nl/#/CBS/nl/dataset/7123slac/table?ts=1584356037989 (accessed May 27, 2020).
26. ZuivelNL. *Dairy in Numbers (in Dutch).* The Hague, Netherlands: ZuivelNL (2019).
27. RIVM. *The Diet of the Dutch Results of the Dutch National Food Consumption Survey 2012-2016.* Bilthoven, Netherlands: National Institute for Public Health and the Environment (2020).
28. RIVM. *Database Environmental Impact of Food.* (2021). Available online at: https://www.rivm.nl/voedsel-en-voeding/duurzaam-voedsel/database-milieubelasting-voedingsmiddelen (accessed January 12, 2021).
29. Blonk Consultants. *Agri-Footprint 5.0 - Part 2: Description of Data.* Gouda, the Netherlands: Blonk Agri-footprint BV (2019).
30. Blonk H, Kool A, Luske B. *Environmental Effects of the Dutch Consumption of Protein Rich Products (In Dutch: Milieueffecten Nederlandse consumptie van eiwitrijke producten).* Gouda, Netherlands: Blonk Environmental Consultants (2008).
31. Van Dooren C, Aiking H, Vellinga P. In search of indicators to assess the environmental impact of diets. *Int J Life Cycle Assess.* (2018) 23:1297–314. doi: 10.1007/s11367-017-1371-2
32. Luske B, Blonk H. *Environmental Impact of Animal By-Products (In Dutch: Milieueffecten van dierlijke bijproducten)*. Gouda, Netherlands: Blonk Consultants (2009).
33. Netherlands Nutrition Centre. *Fact Sheet: The Wheel of Five.* The Hague, Netherlands (2017). Available online at: https://www.voedingscentrum.nl/Assets/Uploads/voedingscentrum/Documents/Professionals/Pers/Factsheets/English (accesed December 18, 2020).
34. Health Council. *Nutritional Guidelines for Energy, Protein, Fats and Digestible Carbohydrates (In Dutch)*. The Hague, Netherlands: Health Council of the Netherlands (2001).
35. Health Council. *Dutch Dietary Guidelines 2015.* The Hague, Netherlands: Health Council of the Netherlands (2015).
36. Brink L, Postma-Smeets A, Stafleu A, Wolvers D. *Guidelines Wheel of Five (In Dutch: Richtlijnen Schijf van Vijf).* The Hague, Netherlands: Voedingscentrum (2016).
37. Judge MM, Pabiou T, Murphy J, Conroy SB, Hegarty PJ, Berry DP. Potential exists to change, through breeding, the yield of individual primal carcass cuts in cattle without increasing overall carcass weight. *J Anim Sci.* (2019) 97:2769–79. doi: 10.1093/jas/skz152
38. Pork Checkoff. *A Typical Market Pig Today*. National Pork Board, Iowa, USA (2017). Available online at: www.porkcheckoff.org (accesed April 2, 2021).
39. van der Peet G, Leenstra F, Vermeij I, Bondt N, Puister L, Van Os J. *Facts and Figures About the Dutch Livestock 2018 (In Dutch: Feiten en cijfers over de Nederlandse veehouderijsectoren 2018)* (No. 1134). Wageningen, Netherlands: Wageningen Livestock Research (2018). doi: 10.18174/464128
40. Dagevos H, Verhoog D, Van Horne P, Hoste P. Meat Consumption per Habitant in the Netherlands 2005-2018 *(In Dutch: Vleesconsumptie per hoofd van de bevolking in Nederland, 2005-2018)*. Wageningen: Wageningen Economic Research (2019). doi: 10.18174/499852
41. Terluin IJ, Kamphuis BM, Oudendag DA, van Leeuwen MGA. *Food Security in the Netherlands During Extraordinary Crises Circumstances (In Dutch:Voedselvoorziening in Nederland onder buitengewone crisisomstandigheden)*. The Hague, Netherlands: Wageningen UR, LEI (2013).
42. Van Dooren C, Aiking H. Defining a nutritionally healthy, environmentally friendly, and culturally acceptable Low Lands Diet. *Int J Life Cycle Assess.* (2016) 21:688–700. doi: 10.1007/s11367-015-1007-3
43. Schneider L, Finkbeiner PM. *Life Cycle Assessment of EU Oilseed Crushing Vegetable Oil Refining - Commissioned by FEDIOL*. Berlin, Germany: Technische Universität Berlin, Chair of Sustainable Engineering (2013).
44. Eshel G, Shepon A, Shaket T, Cotler BD, Gilutz S, Giddings D, et al. A model for "sustainable" US beef production. *Nat Ecol Evol.* (2017) 2:81–5. doi: 10.1038/s41559-017-0390-5
45. Schuurman RWC, Beukers MH, Van Rossum CTM. *Does the Netherlands Eat and Drink According the Food-Based Dietary Guidelines? Results of the National Dietary Survey 2012-2016 (In Dutch: Eet en drinkt Nederland volgens de Richtlijnen Schijf van Vijf? Resultaten van de voedselconsumptiepeiling 2012-2016)*. Bilthoven: RIVM (2020).
46. Ockerman HW, Basu L. *By-products: Edible, for Human consumption, Encyclopedia of Meat Sciences*. 2nd ed. Columbus, OH: The Ohio State University (2014). p. 104–11. doi: 10.1016/B978-0-12-384731-7.00031-3
47. Alao BO, Falowo AB, Chulayo A, Muchenje V. The potential of animal by-products in food systems: production, prospects and challenges. *Sustainability*. (2017) 9:1089. doi: 10.3390/su9071089
48. Vijay V, Pimm SL, Jenkins CN, Smith SJ. The impacts of oil palm on recent deforestation and biodiversity loss. *PLoS ONE*. (2016) 11:e0159668. doi: 10.1371/journal.pone.0159668
49. Van Dooren C, Tyszler M, Kramer GFH, Aiking H. Combining low price, low climate impact and high nutritional value in one shopping basket through diet optimization by linear programming. *Sustainability*. (2015) 7:12837–55. doi: 10.3390/su70912837
50. Broekema R, Tyszler M, Van't Veer P, Kok FJ, Martin A, Lluch H, et al. Future-proof and sustainable healthy diets based on current eating patterns in the Netherlands. *Am J Clin Nutr.* (2020) 112:1338–47. doi: 10.1093/ajcn/nqaa217
51. Dagevos H. Finding flexitarians: current studies on meat eaters and meat reducers. *Trends Food Sci Technol.* (2021) 114:530–9. doi: 10.1016/j.tifs.2021.06.021

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Plant-and Animal-Based Protein Sources for Nutritional Boost of Deep-Fried Dough

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Flaxseed (*Linum usitatissimum* L.) is an oilseed that is used in both industry and food production. Flaxseed contains biologically active compounds including linolenic acid, linoleic acid, and lignans. Flaxseed powder (2.5–10% w/w) and chicken eggs (10–30% v/w) were substituted in cake wheat flour for fried dough (*magwinya*) production. The physicochemical properties of the fried dough were determined and compared with the control. There was a significant ($p < 0.05$) increase in moisture, protein, ash, and hardness and a decrease in fat content of fried dough enriched with egg (FDE) and flaxseed powder (FDFX). Enrichment with 30% (v/w) egg and/or 7.5–10% flaxseed powder resulted in higher protein, oil reduction, and ash contents compared with the rest of the products. With the current interest in plant-based protein, flaxseed is a good choice of flour-based snacks for consumers without compromise in nutrition.

Keywords: flaxseed powder, chicken egg, *magwinya*, plant-based protein, animal protein, enrichment

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INTRODUCTION

Protein is formed from amino acids, which are the building blocks of muscle mass that are mostly found in animal products and some plant products. One (1) gram of protein equals 4 calories and is composed of amino acids formed by nitrogen, carbon, hydrogen, oxygen, and sulfur. In bakery products, protein plays a role in the texture of the food by forming gels, stabilizing foams, and emulsions. Chicken eggs are considered human foods for a long time and they are protein-rich containing high-quality nutrients. It is a source of iron, riboflavin, folate, and vitamin B12, D, and E, and contains all nine essential amino acids (Lesnierowski and Stangierski, 2018). Eggs function to enhance the texture and flavor of food products (Vaclavik and Christian, 2014). The egg white (albumen) is an aqueous, faintly straw-tinted, gel-like liquid, consisting of 87.72% water, 0.85% carbohydrates, 10.82% crude protein, 0.19% fat and 0.42% ash (Li, 2006; Réhault-Godbert et al., 2019). Egg yolk is a fat-in-water emulsion containing 50% dry weight; consisting of 65% lipids, 31% proteins and 4% carbohydrates, vitamins, and minerals (Kovacs-Nolan et al., 2005). In dough production, eggs are used to bind other ingredients and strengthen the dough (Indrani and Rao, 2007).

Flaxseed (*Linum usitatissimum* L.) is an ancient plant that originated in Asia and is now used in both industry and food production (Marie and Ivan, 2017). Flaxseed contains about 8% crude protein, thereby making it a functional food ingredient (Hussain et al., 2008). Flaxseed has found use in many plant-based foods like an egg replacer due to its rich protein (globulin and albumin) content—speaking to its functionality in baked goods (Bernacchia et al., 2014). Flaxseed is rich in fiber (22–26%) with cellulose, mucilage gums, and lignans as chief fractions. Mucilage gum is a form of polysaccharide that becomes gelatinous on mixing with liquids (Tufail et al., 2020). Flaxseed is also highly acclaimed for its omega-3-fatty acid for plant-based consumers. Of all lipids in flaxseed, 53% are α-linolenic acid (ALA), 17% linoleic acid (LA), and 19% oleic acid (Gutte et al., 2015).

Magwinya, also known as "fat cake," is a deep-fried dough product mostly consumed in sub-Saharan Africa (Kearney et al., 2011). The snack is prepared from deep-frying a fermented mixture of refined wheat flour, sugar, salt, yeast, and water. These ingredients provide limited nutrients needed by the body to function well. Eggs are not usually included in the production process in Southern Africa. However, eggs are used in the Belgian version—*oliebollen* (Ghijselings, 2019). This means that the snack is low in protein. It is characterized as an oily, moist, sweet/salty, crispy golden-brown confectionary. Fat cake can be cut open and stuffed with cheese, mince, and jam or served with fried chips and fish, either as a breakfast with tea or a snack with a soft drink (Onipe et al., 2019). Moreover, primary school learners and the low-income population are the major consumers of this snack (Onipe et al., 2019). Given the burden of malnutrition that plagues low-and middle-income populations of the world, children suffer the most, one of which is protein deficiency (Otiti and Allen, 2021). Egg is a cheap and readily available source of protein for non-vegans, while flaxseed powder is a cheaper protein alternative for plant-based consumers. Hence, this research investigated the effect of egg and flaxseed on the physicochemical properties of fried dough (*magwinya*).

MATERIALS AND METHODS

Production of Fried Products

Cake wheat flour, yeast, brown sugar, salt, sunflower cooking oil, flaxseed powder, and chicken eggs were locally sourced from a supermarket in Limpopo Province, South Africa. Concentrated sulfuric acid, selenium tablet, sodium hydroxide pellets, boric acid, methyl red, and petroleum ether (40–60°C) were of analytical grade and were used in protein and fat analysis. The chemicals were purchased from Sigma-Aldrich. Fried dough products were produced using the traditional method described by Kwinda et al. (2018) with modifications.

Egg-Enriched Fried Dough (FDE)

Water and egg mixtures (v/v) were prepared in ratios shown in **Table 1**. The mixtures were added to dry ingredients (cake wheat flour—100 g, sugar—15 g, yeast—1 g, and salt—1 g). Wet and dry ingredients were mixed until a homogeneous sticky dough was formed.

Flaxseed-Enriched Fried Dough (FDFX)

Wheat flour was composited with flax seed powder (**Table 1**). 100 grams each of the composite flour was each combined with yeast (1 g), salt (1 g), and sugar (15 g). To each product, 100 ml of lukewarm water (36.5 to 40.5°C) was added to form a homogeneous sticky dough.

The dough products were fermented at ambient temperature (30°C) and deep-fried in sunflower oil at 180°C for 5 min each. The fried dough was drained and cooled to 25°C on absorbent paper until analysis was done. The control product had no eggs or flaxseed powder in its formulation. The frying process and experiments were carried out in triplicate batches.

Methods

Fried dough products were cooled down to ambient temperature (25°C), and weights of three products from the three frying batches were collected using a digital weighing balance (Onipe et al., 2018). To ensure data homogeneity, samples with similar weights were randomly selected for the subsequent measurements.

Hardness

The fried products were cooled for 30 min after frying and the hardness was measured in triplicate using the approved method 74-09.01 of American Association of Cereal Chemists, 1999. The texture analyzer (TA. XT Plus, Stable Micro Systems Ltd, Godalming, UK) fitted with a 5-kg load cell and a 35-mm cylindrical probe was used for the testing. A return-to-start test was used for measurement with the following settings: 40% strain, 2 mm/s, and 1 mm/s test and post-test speeds, respectively. The peak positive force (g) in the force-deformation curve was recorded as the hardness value (AACC, Onipe et al., 2018).

Color Determination

Color analysis was performed using the method of Ndlala et al. (2019) in a ColorFlex 45/0 Spectrophotometer (Hunterlab, Reston, USA) with illuminant D65 and a 10°C observer. Three crust and crumb of fried dough products were analyzed for L* (luminosity), a* (opposition of colors green and red), and b* (opposition of colors blue and yellow). Chroma (C*), hue (h), and total color difference (ΔE) were calculated using Eqs 1–3.

$$\text{Chroma} = \sqrt{a^2 + b^2} \quad (1)$$

$$\text{Hue} = \tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

$$\Delta E = \sqrt{\left[(L - Lc)^2 + (a - ac)^2 + \left(b - bc\right)^2\right]} \quad (3)$$

where L* represents lightness from black to white (0–100); a* measures red (+) to green (–); b* measures yellow (+) to blue (–), whereas Lc, ac, and bc represent the color values for the control fried dough (without egg or flaxseed powder). ΔE represents the degree of the overall color change of a sample in comparison to color values (Lc, ac, and bc) of the control (Onipe et al., 2018).

Physicochemical Analysis

Moisture, oil, and ash contents were determined in triplicate using the approved methods 44-15, 30-25.01, and 08-01.01 of American Association of Cereal Chemists (1999), respectively. The products were dried, pulverized, and tested for protein content using the Kjeldahl method, according

TABLE 1 | Formulations for fried dough products.

Products	Water (ml)	Egg(ml)	Flaxseed powder (g)	Cake wheat flour (g)
Control	100	–	–	100
FDE10	90	10	–	100
FDE20	80	20	–	100
FDE30	70	30	–	100
FDFX2.5	100	–	2.5	97.5
FDFX5.0	100	–	5.0	95
FDFX7.5	100	–	7.5	92.5
FDFX10	100	–	10	90

Yeast (1 g), salt (1 g), and sugar (15 g) were uniform in all the products. FDE = egg-enriched fried dough, FDFX = flaxseed powder-enriched fried dough.

to method 955.04 of the Association of Official Analytical Chemists (1995). The percentage nitrogen (N_2) in protein was multiplied by a factor of 6.25 to get protein content. Total carbohydrate was determined by the difference method using Equation 4.

$$\%\text{Carbohydrate} = 100 - (\%\text{fat} + \%\text{protein} + \%\text{ash} + \%\text{moisture}) \quad (4)$$

Statistical Analysis

Data were analyzed by one-way analysis of variance. Significant differences ($p < 0.05$) between means were determined by Duncan's multiple range tests (Duncan, 1955). The results were analyzed using a Statistical Package for Social Sciences (SPSS version 23).

RESULTS AND DISCUSSION

Hardness of Egg-and Flaxseed-Enriched Fried Dough

The values for the hardness of FDE and FDFX products ranged from 1,343.37 to 1,605.24 g and 1,551.72 to 1,975.35 g, respectively (**Figure 1**). The hardness of FDE products was significantly higher than the control, while that of FDFX was significantly lower than the control. Meaning flaxseed caused the fried dough to be softer in comparison to chicken eggs. The results of FDFX showed no linear trend, but a significant reduction ($p < 0.05$) was noted at 2.5, 7.5, and 10%. The differences in the hardness of FDE and FDFX could be accounted for by the variation of water in their formulations. For the FDFX products, the flaxseed powder was worked into the flour, thereby reducing the gluten content (which traps a significant amount of water in a dough matrix) and the amount of water available in the dough. Moreover, the presence of mucilage gum in the flaxseed powder could have caused gas and moisture retention, causing less evaporation of water out of the food during frying, thereby accounting for the softness of FDFX compared to FDE products (Budžaki and Šeruga, 2005; Tufail et al., 2020). Egg yolk is known to improve the richness and tenderness of dough and eating quality, while albumen provides baked products with a good volume and texture (Mine and D'Silva, 2008; Zhao et al., 2010; Van Steertegem et al., 2013).

Color Profile of Egg-and Flaxseed-Enriched Fried Dough

Crust and Crumb Color of Egg-Enriched *Magwinya*

The crust color profile of FDE products is presented in **Table 2**. The lightness (L*), redness (a*), and yellowness values for FDE were in the ranges 31.74–33.03, 14.56–17.45, and 17.60–23.71, respectively. The addition of eggs influenced the crust darkening as observed in the reduction of L* values with an increase in egg content. This is because amino acids in the egg react with the reducing sugars through the process of Maillard reaction that produces brown color, flavor, and aroma during thermal processing of food (Fayle and Gerrard, 2002; Calderón-Domínguez et al., 2005; Onipe et al., 2018). The redness and yellowness of FDE decreased with an increased egg addition. The total color change is the degree of the overall color change of a sample in comparison to the color values of the control (Pathare et al., 2013). The chroma, hue angle, and color change of the FDE decreased with the increase in egg addition compared to control. Hue angle qualitatively describes a product's typical color in terms of real colors such as red or green (Ndlala et al., 2019). The crumb lightness, redness, yellowness, chroma, and ΔE of FDE products were significantly higher than those of the control. As expected, the crumb was lighter than the crust because the latter is exposed to the highest heat during frying. The increase in yellowness of FDE compared to control is highly influenced by the yellow color of the egg yolk.

Crust and Crumb Color Profile of Flaxseed-Enriched Fried Dough

The values of crust color properties for the crust of *magwinya* were within the following range: L* (41.44–55.26), a* (4.44–5.43), b* (11.54–17.29), Chroma (12.45–18.12), hue angle (67.97–72.57), and ΔE (12.99–19.12). Compared to the control, the results of crust color (**Table 3**) indicate a decrease in the lightness, redness, yellowness, and chroma values, and an increase in hue and total color change. Similarly, crumb lightness, yellowness chroma, and hue decreased significantly in FDFX products relative to the control. The redness of the crumb, however, increased markedly—a direct impact of the dark hue of flaxseed powder (**Figure 2**). Coupled with intense Maillard reaction, these variations in crust color relative to the control are linked to the dark color (brown) of the flaxseed powder. Marpalle et al. (2014)

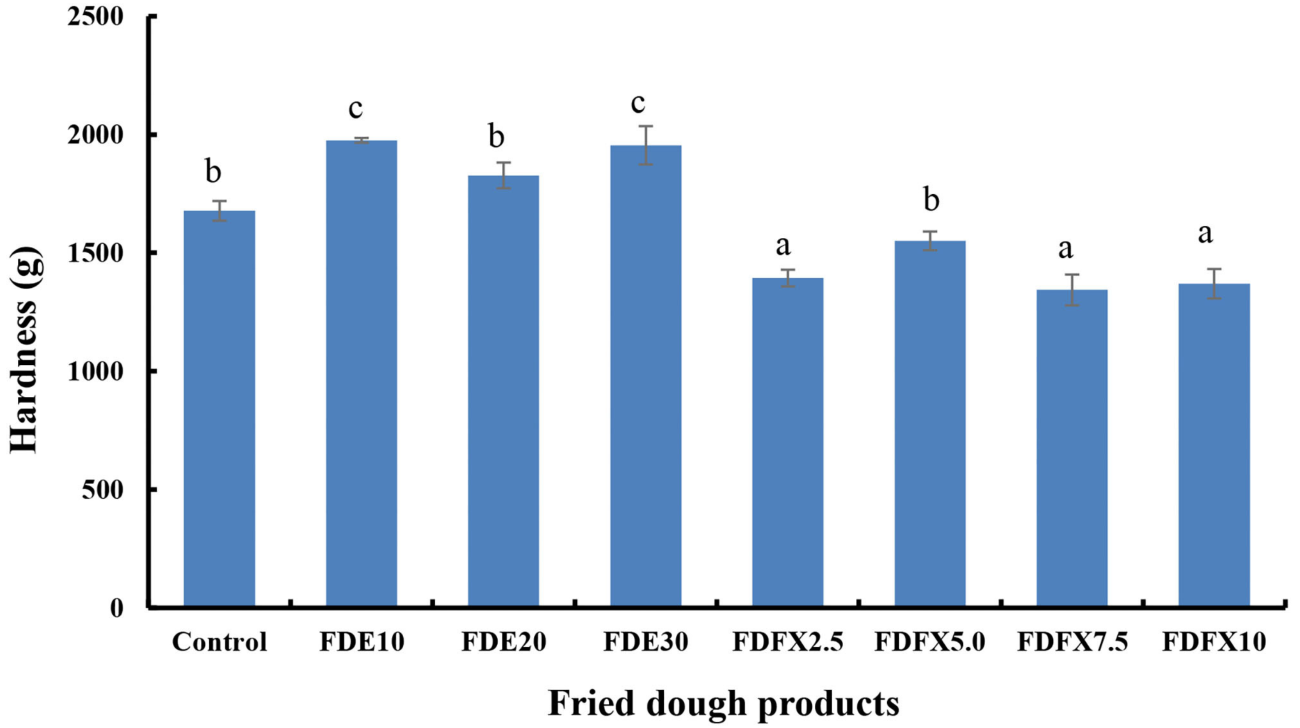

FIGURE 1 | Hardness (g) of fried dough products. Results were presented as mean ± standard deviation ($n = 3$). Superscripts were assigned to show significant differences ($p < 0.05$) with an increase in egg/flaxseed powder using Duncan multiple range test. FDE = egg-enriched fried dough, FDFX = flaxseed powder-enriched fried dough.

TABLE 2 | Color profile of egg-enriched fried dough.

Fried products	Control	FDE10	FDE20	FDE30
Crust				
L*	34.51 ± 1.42^{c}	35.53 ± 0.40^{d}	31.74 ± 1.45^{a}	32.01 ± 1.55^{b}
a*	17.44 ± 1.89^{c}	16.86 ± 1.94^{b}	16.77 ± 1.57^{a}	17.79 ± 3.59^{c}
b*	21.49 ± 4.13^{c}	23.71 ± 4.83^{d}	20.08 ± 1.81^{b}	17.60 ± 10.37^{a}
Chroma	27.70 ± 4.32^{c}	29.45 ± 5.05^{d}	25.55 ± 2.39^{b}	23.03 ± 10.34^{a}
Hue	50.70 ± 2.77^{b}	53.37 ± 2.39^{c}	51.84 ± 0.28^{b}	47.77 ± 8.82^{a}
ΔE	–	3.74 ± 1.11^{a}	5.25 ± 1.38^{b}	8.88 ± 3.43^{c}
Crumb				
L*	63.01 ± 1.11^{a}	66.91 ± 3.35^{b}	68.39 ± 2.45^{c}	69.35 ± 2.70^{c}
a*	2.29 ± 1.12^{a}	2.89 ± 0.53^{b}	3.37 ± 0.18^{c}	3.74 ± 0.12^{c}
b*	21.41 ± 5.60^{a}	25.91 ± 2.09^{b}	29.34 ± 1.42^{c}	30.54 ± 1.23^{d}
Chroma	21.54 ± 5.68^{a}	26.08 ± 2.13^{b}	29.53 ± 1.42^{c}	30.77 ± 1.28^{d}
Hue	83.55 ± 1.62^{a}	83.68 ± 0.71^{a}	83.44 ± 0.35^{a}	83.01 ± 0.19^{a}
ΔE	–	8.86 ± 7.76^{a}	12.49 ± 11.25^{b}	13.94 ± 11.39^{c}

Results were presented as mean ± standard deviation (n = 3). Superscripts (a, b, c, d) were assigned to show significant difference (p < 0.05) with an increase in egg concentration using Duncan's multiple range test. FDE = egg-enriched fried dough.

reported a similar trend in bread enriched with flaxseed. Onipe et al. (2019) reported that wheat bran with medium particle size had a similar effect on the crust and crumb color profile of fried dough. Care must be taken to avoid consumers being put off by the dark color impacted by the brown flaxseed. The decrease in lightness and yellowness with an increase in FP level agrees with the results obtained by Codină et al. (2016) on bread.

TABLE 3 | Color profile of flaxseed-enriched fried dough.

Fried products	Control	FDFX2.5	FDFX5.0	FDFX7.5	FDFX10
Crust					
L*	34.51 ± 1.42^{d}	32.49 ± 0.09^{b}	32.80 ± 0.23^{b}	31.81 ± 0.16^{a}	33.55 ± 0.20^{c}
a*	17.44 ± 1.89^{d}	8.30 ± 0.20^{b}	9.06 ± 0.51^{c}	6.55 ± 0.25^{a}	8.71 ± 0.59^{bc}
b*	21.49 ± 4.13^{d}	14.30 ± 0.27^{b}	14.68 ± 0.22^{b}	13.52 ± 0.38^{a}	15.94 ± 0.42^{c}
Chroma	27.70 ± 4.32^{d}	9.35 ± 0.06^{b}	10.19 ± 0.55^{bc}	7.44 ± 0.26^{a}	10.55 ± 0.72^{c}
Hue	50.70 ± 2.77^{a}	59.87 ± 2.01^{b}	58.34 ± 0.23^{b}	64.16 ± 2.90^{d}	61.37 ± 0.36^{c}
ΔE	–	12.05 ± 0.23^{b}	11.17 ± 0.70^{ab}	13.98 ± 0.20^{c}	10.67 ± 0.84^{a}
Crumb					
L*	63.01 ± 1.11^{a}	55.26 ± 1.30^{d}	49.77 ± 1.54^{c}	45.86 ± 0.24^{b}	41.44 ± 0.34^{a}
a*	2.29 ± 1.12^{a}	5.43 ± 0.50^{c}	4.44 ± 0.07^{b}	4.66 ± 0.40^{b}	4.91 ± 0.07^{bc}
b*	21.41 ± 5.60^{d}	17.29 ± 1.60^{c}	13.77 ± 0.68^{b}	11.54 ± 1.23^{a}	12.67 ± 0.45^{ab}
Chroma	21.54 ± 5.68^{d}	18.12 ± 1.68^{c}	14.47 ± 0.66^{b}	12.45 ± 1.29^{a}	13.59 ± 0.43^{ab}
Hue	83.55 ± 1.62^{c}	72.57 ± 0.07^{b}	72.09 ± 0.72^{b}	67.97 ± 0.43^{a}	68.82 ± 0.68^{a}
ΔE		12.99 ± 0.92^{a}	19.12 ± 1.23^{b}	23.53 ± 0.76^{c}	26.46 ± 0.10^{d}

Results were presented as mean ± standard deviation (n = 3). Superscripts (a, b, c, d, ab, bc) were assigned to show significant difference (p < 0.05) with an increase in flaxseed powder concentration using Duncan multiple range test. FDFX = flaxseed powder-enriched fried dough.

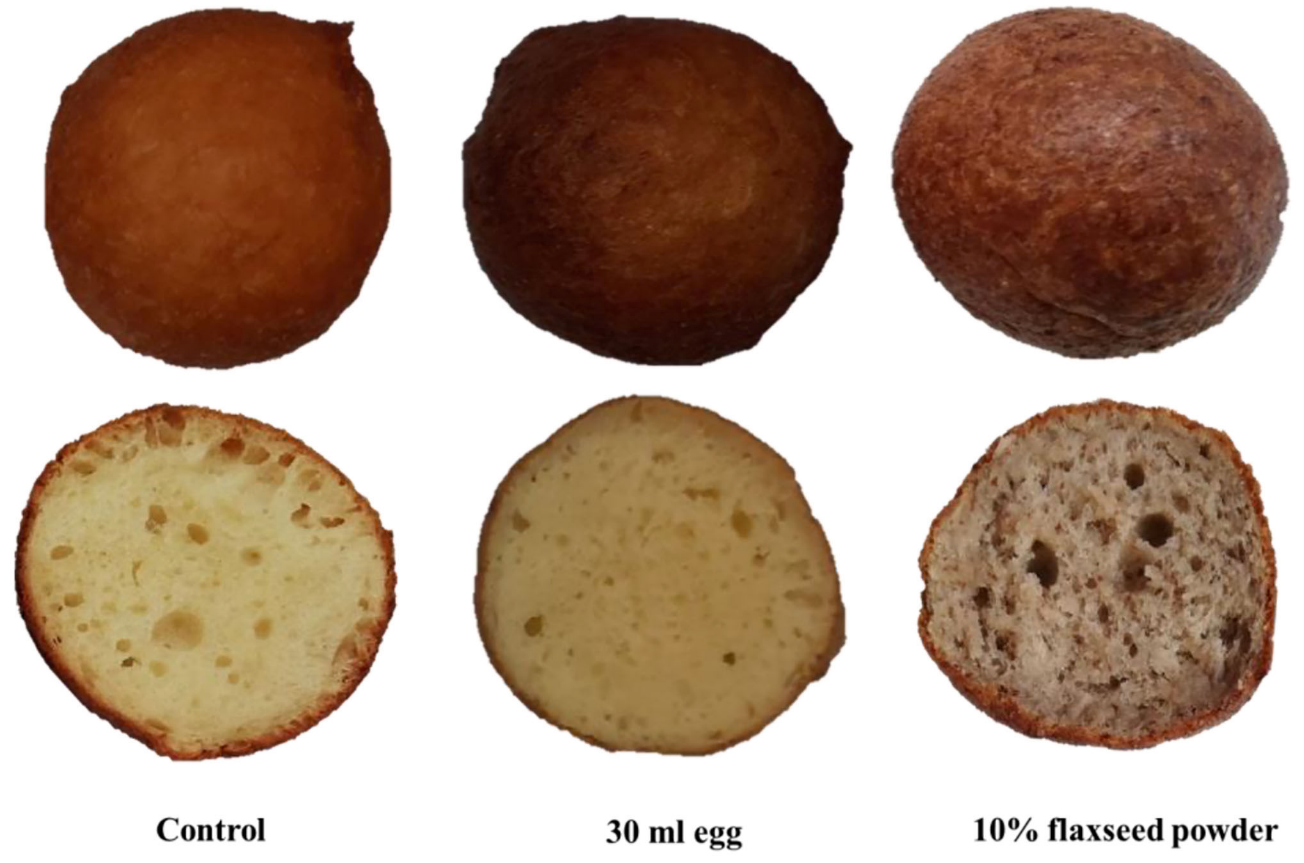

FIGURE 2 | Fried cereal-dough products.

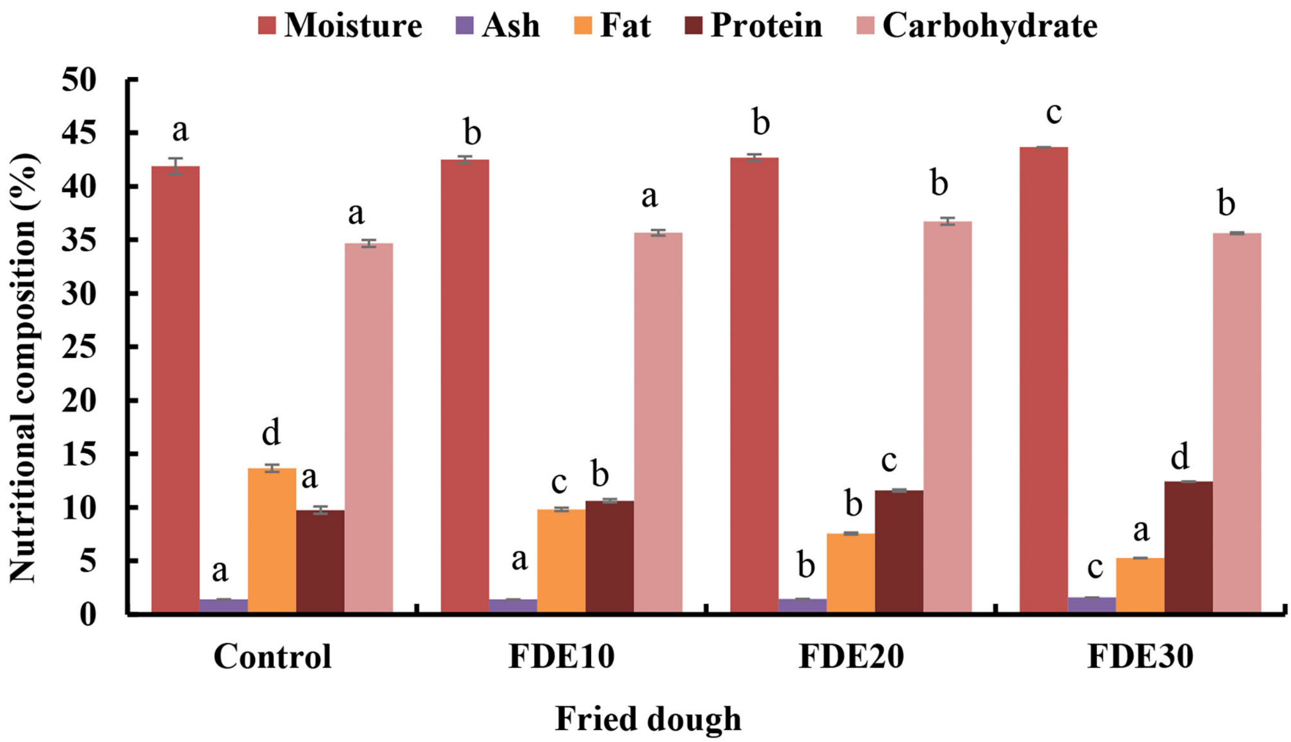

FIGURE 3 | Nutritional composition of egg-enriched fried dough. Results were presented as mean ± standard deviation ($n = 3$). Superscripts were assigned to show significant differences ($p < 0.05$) with an increase in egg in similar bars using Duncan multiple range test. FDE = egg-enriched fried dough.

Moisture Content of Egg-and Flaxseed-Enriched Fried Dough

The mean moisture values of FDE ranged from 42.49 to 45.10% (**Figure 3**) and that of FDFX ranged from 42.00 to 43.33% and were slightly higher than the control at 41.89% (**Figure 4**). The increased moisture content of FDE can be attributed to the ability of eggs to bind with other ingredients during the formation of dough. The results showed that the incorporation of eggs improved the moisture content of *magwinya*, albeit small. There was no significant difference ($p < 0.05$) in the moisture content of the products with different amounts of flaxseed powder, but the moisture content of control was significantly low. The increase in moisture content of FDFX products may be due to the fiber content of flaxseed powder, which increased high moisture retention (Ganorkar and Jain, 2014). Similar results were reported by Xu et al. (2014) where the moisture content of bread markedly increased with the increase in flaxseed flour.

Ash Content of Egg-and Flaxseed-Enriched Fried Dough

The ash content of FDE ranged from 1.40 to 1.58% (**Figure 3**) and FDFX ranged from 1.56 to 1.97% (**Figure 4**) and were all significantly higher than control (1.41%). An increasing linear trend ($p < 0.05$) was observed for the ash content of FDE and FDFX products with an increase in egg and flaxseed powder concentration. The increase in ash content in both FBE and FDE shows that egg and flaxseed powder increased the mineral content of fried dough compared to control. This is because of the high mineral content of eggs (Haytowitz and Pehrsson, 2018) and flaxseeds (Codină et al., 2016). Masoodi and Bashir (2012) reported a 69% increase in the ash content of biscuits enriched with 10% flaxseed powder.

Fat Content of Egg-and Flaxseed-Enriched Fried Dough

The fat content of FDE products ranged from 5.27 to 9.82% (**Figure 3**), while FDFX ranged from 6.08 to 9.41% (**Figure 4**). The fat content of FDE and FDFX products was significantly lower than control (13.66%). During deep-frying, the heat evaporates moisture out of the products, while oil penetrates inside the products (Krokida et al., 2000; Budžaki and Šeruga, 2005). Due to the consistency of the control dough, large gas cells are formed during the fermentation process, thereby creating more pores for oil uptake during frying and higher fat content in the control products compared to other products (Ivorra et al., 2014). Egg binds the ingredients together during dough mixing, thereby increasing the dough strength and its elasticity, which may have impeded moisture loss and contributed to the reduced fat content in FDE products (Indrani and Rao, 2007; Van Steertegem et al., 2013). The least fat contents for FDE and FDFX were recorded at 30% egg and 10% flaxseed powder addition, showing that both additives reduced the fat content of the fried dough. Contrary to what other researchers have reported that flaxseed increased the fat content of other products

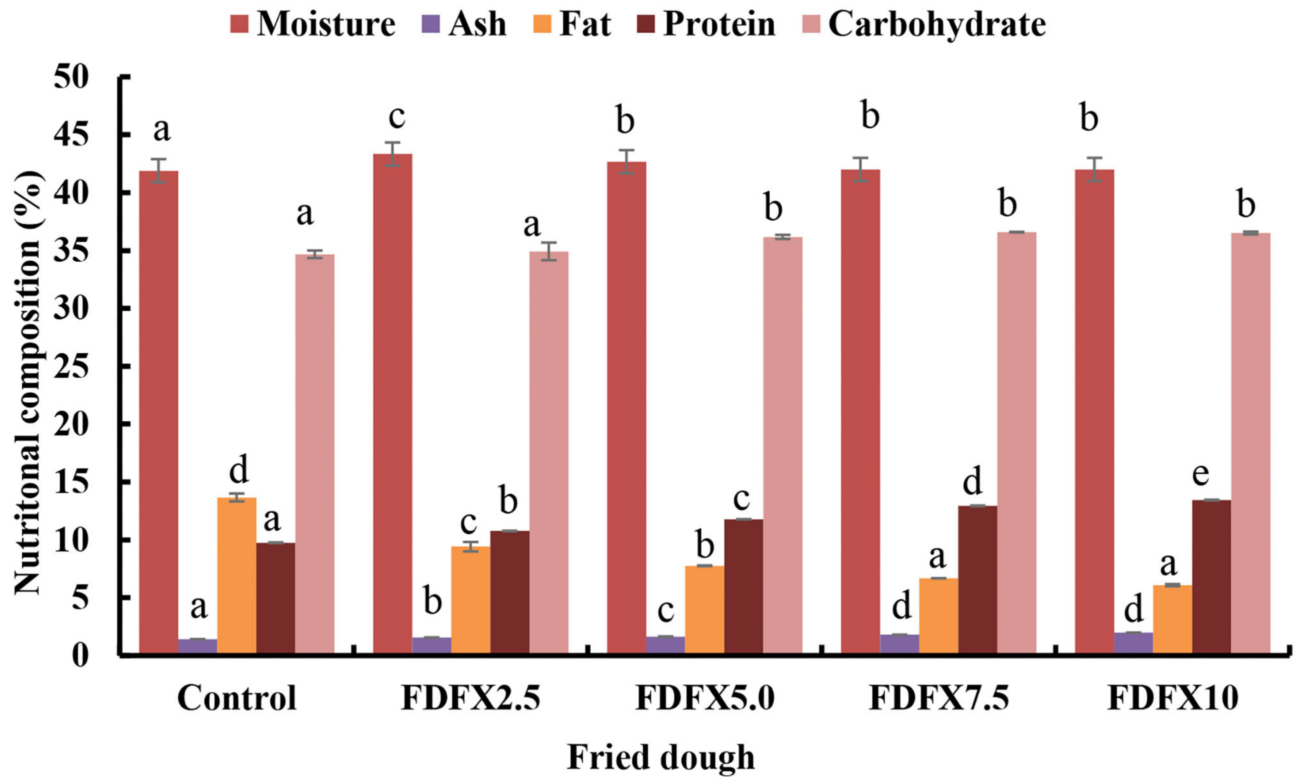

FIGURE 4 | Nutritional composition of flaxseed-enriched fried dough. Results were presented as mean ± standard deviation ($n = 3$). Superscripts were assigned to show significant differences ($p < 0.05$) with an increase in egg in similar bars using Duncan multiple range test. FDFX = flaxseed-enriched fried dough.

like cookies and bread (Masoodi and Bashir 2012), the use of flaxseed in our product resulted in reduced fat content. This may be because of the high-water retention capacity of flaxseed, which may have led to a minimal replacement of water with oil, hence the decrease in fat content (Mellema, 2003; Kwinda et al., 2018).

Protein Content of Egg-and Flaxseed-Enriched Fried Dough

The protein contents of the FDE products ranged from 10.63 to 12.42% (**Figure 3**), and FDFX ranged from 10.77 to 13.44% (**Figure 4**) and were all significantly higher than the control (9.75%). The highest protein values were found in FDE30 and FDFX10. This shows that the addition of eggs and/or flaxseed increased the protein content of *magwinya.* This is because an egg contains about 12.5% protein from both albumen and yolk (Seuss-Baum and Francoise, 2011) and the rich protein content of flaxseed (Hussain et al., 2008; Tufail et al., 2020). Other authors have reported a significant increase in the protein content of biscuits (Masoodi and Bashir, 2012) and cookies (Kaur et al., 2019) enriched with flaxseed. The protein contents of whole wheat-based bread increased with the increased addition of chia seed flour (Sayed-Ahmad et al., 2018).

Carbohydrate Content of Egg-and Flaxseed-Enriched Fried Dough

As shown in **Figures 3**, **4** carbohydrate content increased significantly with an increase in egg addition from 20% and flaxseed powder from 5% flaxseed. The polysaccharide content of flaxseed could have contributed to this increase. Since carbohydrate content was calculated using the difference method, the decrease in fat content could have encouraged the increase in the percentage of carbohydrates. Marpalle et al. (2014) reported a significant decrease in carbohydrate percentage with an increase in flaxseed flour on functional bread.

CONCLUSION

Supplementing the formulation of fried dough with eggs and flaxseed powder positively influenced the physicochemical properties of the fried dough products. Increased levels of egg and flaxseed powder increased the moisture, ash, and protein content while reducing fat content. The protein contents of FDE and FDFX products were comparable, but the highest level was reported at 10% flaxseed supplementation. Flaxseed powder had no significant impact on the hardness of the fried dough, but it decreased the lightness, redness, and yellowness of the product. The effect on color may repel consumers, but a consumer acceptability test is recommended to confirm or reject this assumption. Further research is recommended on the impact of egg and flaxseed powder on the microstructure, mineral content, and rheological properties of the dough. The findings of this research show that nutritious low-fat fried dough can be produced from plant and animal protein sources to cater for consumers on both diets.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

OOO and AIOJ supervised the project and defined the objective of the article. NM and KR collected and analyzed the data. OOO drafted and AIOJ proofread the manuscript. All authors participated in the interpretation of results.

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REFERENCES

American Association of Cereal Chemists (1999). *Approved methods of American Association of Cereal Chemists*. Available online at http://methods.aaccnet.org/toc.aspx (accessed September 15, 2020).

Association of Official Analytical Chemists (AOAC) (1995). *Official Methods of Analysis, 16th Edn*. Gaithersburg, MD: AOAC International.

Bernacchia, R., Preti, R., and Vinci, G. (2014). Chemical composition and health benefits of flaxseed. *Austin J. Nutr. Food Sci.* 2, 1045–1053.

Budžaki, S., and Šeruga, B. (2005). Moisture loss and oil uptake during deep-fat frying of "Kroštula" dough. *Eur. Food Res. Technol.* 220, 90–95. doi: 10.1007/s00217-004-1058-3

Calderón-Domínguez, G., Farrera-Rebollo, R., Arana-Errasquín, R., and Mora-Escobedo, R. (2005). The effect of varying the mixing formula on the quality of a yeast sweet bread on the process conditions, as studied by surface response methodology. *Int. J. Food Sci. Technol.* 40, 157–164. doi: 10.1111/j.1365-2621.2004.00926.x

Codină, G. G., Mironeasa, S., and Todosi-Sănduleac, E. (2016). Studies regarding the influence of brown flaxseed flour addition in wheat flour of very good quality for bread making on bread quality. *Bull. UASVM Food Sci. Technol.* 73, 70–76. doi: 10.15835/buasvmcn-fst:12148

Duncan, D. B. (1955). Multiple range and multiple *F* tests. *Biometrics* 11, 1–42. doi: 10.2307/3001478

Fayle, S. E., and Gerrard, J. A. (2002). *The Maillard Reaction*. Cambridge: Royal Society of Chemistry.

Ganorkar, P. M., and Jain, R. K. (2014). Effect of flaxseed incorporation on physical, sensorial, textural and chemical attributes of cookies. *Int. Food Res. J.* 21, 1515–1521.

Ghijselings (2019). *Physicochemical properties and hyperspectral imaging of fried cereal doughs–magwinya and oliebollen* (Bachelor's thesis). UC Lueven-Limburg, Brabant, Belgium.

Gutte, K. B., Sahoo, A. K., and Ranveer, R. C. (2015). Bioactive components of flaxseed and its health benefits. *Int. J. Pharm. Sci. Rev. Res.* 31, 42–51.

Haytowitz, D. B., and Pehrsson, P. R. (2018). USDA's National Food and Nutrient Analysis Program (NFNAP) produces high-quality data for USDA food composition database: two decades of collaboration. *Food Chem.* 238, 134–138. doi: 10.1016/j.foodchem.2016.11.082

Hussain, S., Anjum, F. M., Butt, M. S., and Sheikh, M. A. (2008). Chemical composition and functional properties of flaxseed (*Linum usitatissimum*) flour. *Sarhad J. Agric.* 24, 649–653.

Indrani, D., and Rao, G. V. (2007). Rheological characteristics of wheat flour dough as influenced by ingredients of parotta. *J. Food Eng.* 79, 100–105. doi: 10.1016/j.jfoodeng.2006.01.033

Ivorra, E., Amat, S. V., Sánchez, A. J., Barat, J. M., and Grau, R. (2014). Continuous monitoring of bread dough fermentation using a 3D vision structured light technique. *J. Food Eng.* 130, 8–13. doi: 10.1016/j.jfoodeng.2013.12.031

Kaur, P., Sharma, P., Kumar, V., Panghal, A., Kaur, J., and Gat, Y. (2019). Effect of addition of flaxseed flour on phytochemical, physicochemical, nutritional, and textural properties of cookies. *J. Saudi Soc. Agric. Sci.* 18, 372–377. doi: 10.1016/j.jssas.2017.12.004

Kearney, J., Oldewage-Theron, W., and Napier, C. (2011). Development and processing of a novel food product for a school feeding project in South Africa. *Afr. J. Hosp. Tour. Leis*. 1, 4–7.

Kovacs-Nolan, J., Phillips, M., and Mine, Y. (2005). Advances in the value of eggs and egg components for human health. *Review. J. Agric. Food Chem*. 53:8421. doi: 10.1021/jf050964f

Krokida, M. K., Oreopoulou, V., and Maroulis, Z. B. (2000). Effect of frying conditions on shrinkage and porosity of fried potatoes. *J. Food Eng*. 43, 147–154. doi: 10.1016/S0260-8774(99)00143-0

Kwinda, O., Onipe, O. O., and Jideani, A. I. O. (2018). The effect of oat bran and psyllium husk fibre on oil reduction and some physicochemical properties of *magwinya*–a deep-fried dough, CyTA. *J. Food* 16, 247–254. doi: 10.1080/19476337.2017.1389991

Lesnierowski, G., and Stangierski, J. (2018). What's new in chicken egg research and technology for human health promotion? A review. *Trends Food Sci. Technol.* 71, 46–51. doi: 10.1016/j.tifs.2017.10.022

Li, S. J. (2006). Structural details at the active site of hen egg-white lysozyme with di-and trivalent metal ions. *Biopolymers* 81, 1196–1199. doi: 10.1002/bip.20367

Marie, H., and Ivan, Š. (2017). Rheological Characteristics of composite flour with linseed fibre–relationship to bread quality. *Czech J. Food Sci*. 35, 424–431. doi: 10.17221/450/2016-CJFS

Marpalle, P., Sonawane, S. K., and Arya, S. S. (2014). Effect of flaxseed flour addition on physicochemical and sensory properties of functional bread. *LWT-Food Sci. Technol.* 58, 614–619. doi: 10.1016/j.lwt.2014.04.003

Masoodi, L., and Bashir, V. (2012). Fortification of biscuit with flaxseed: biscuit production and quality evaluation. *IOSR J. Environ. Sci. Toxicol. Food Technol.* 1, 6–9. doi: 10.9790/2402-0150609

Mellema, M. (2003). Mechanism and reduction of fat uptake in deep-fat fried foods. *Trends Food Sci. Technol*. 14, 364–373. doi: 10.1016/S0924-2244(03)00050-5

Mine, Y., and D'Silva, I. (2008). "Bioactive components in egg white," in *Egg Bioscience and Biotechnology*, ed Y. Mine (Hoboken, NJ: Wiley and Sons publication), 141–184. doi: 10.1002/9780470181249.ch4

Ndlala, F. N., Onipe, O. O., Mokhele, T. M., Anyasi, T. A., and Jideani, A. I. O. (2019). Effect of wheat bran incorporation on the physical and sensory properties of a South African cereal fried dough. *Food* 8, 559–573. doi: 10.3390/foods8110559

Onipe, O. O., Beswa, D., and Jideani, A. I. O. (2019). The socioeconomic benefits, production and consumption statistics of *magwinya* in Limpopo province, South Africa. *Afr. J. Food, Agric. Nutr. Dev*. 19, 15007–15028. doi: 10.18697/ajfand.87.18030

Onipe, O. O., Beswa, D., Jideani, V. A., and Jideani, A. I. O. (2018). Optimization of processing conditions for oil reduction of *magwinya* (a deep-fried cereal dough). *Afr. J. Sci. Technol. Innov. Dev*. 10, 209–218. doi: 10.1080/20421338.2018.1440920

Otiti, M. I., and Allen, S. J. (2021). Severe acute malnutrition in low- and middle-income countries. *Paediatr. Child Health* 31, 301–307. doi: 10.1016/j.paed.2021.05.001

Pathare, P. B., Opara, U. L., and Al-Said, F. A.-J. (2013). Color measurement and analysis in fresh and processed foods: a review. *Food Bioproc. Tech.* 6, 36–60. doi: 10.1007/s11947-012-0867-9

Réhault-Godbert, S., Guyot, N., and Nys, Y. (2019). The golden egg: nutritional value, bioactivities, and emerging benefits for human health. *Nutrition* 11:684. doi: 10.3390/nu11030684

Sayed-Ahmad, B., Talou, T., Straumite, E., Sabovics, M., Kruma, Z., Saad, Z., et al. (2018). Evaluation of nutritional and technological attributes of whole wheat-based bread fortified with chia flour. *J. Food* 7:135. doi: 10.3390/foods70 90135

Seuss-Baum, I., and Francoise, N. (2011). "The nutritional quality of eggs," in *Improving the Safety and Quality of Eggs and Egg Products*, eds F. van Immerseel, F. Y. Nys, and M. Bain (Cambridge, GB: Woodhead Publishing Ltd), 201–236. doi: 10.1533/9780857093929.3.201

Tufail, T., Riaz, M., Arshad, M. U., Gilani, S. A., Ain, H. B. U., Khursheed, T., et al. (2020). Functional and nutraceutical scenario of flaxseed and sesame: a review. *Int. J. Biosci.* 17, 173-190. doi: 10.12692/ijb/17.3.1 73-190

Vaclavik, V. A., and Christian, E. W. (2014). "Eggs and egg products," in *Essentials of Food Science*, ed D. R. Heldman (New York, NY: Springer), 179–199. doi: 10.1007/978-1-4614-9138-5_10

Van Steertegem, B., Pareyt, B., Brijs, K., and Delcour, J. A. (2013). The effects of fresh eggs, egg white, and egg yolk, separately and in combination with salt, on mixogram properties. *Cereal Chem*. 90, 269–272. doi: 10.1094/CCHEM-11-12-0160-N

Xu, Y., Hall, I. I. I., C. A., and Manthey, F. A. (2014). Effect of flaxseed flour on rheological properties of wheat flour dough and bread characteristics. *J. Food Res*. 3, 83-91. doi: 10.5539/jfr.v3n6p83

Zhao, X., Shi-Jian, D., Tao, G., Xu, R., Wang, M., Reuhs, B., et al. (2010). Influence of phospholipase A2 (PLA2)-treated dried egg yolk on wheat dough rheological properties. *LWT-Food Sci. Tech.* 43, 45–51. doi: 10.1016/j.lwt.2009.06.027

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Dietary Salt Intake and Gastric Cancer Risk: A Systematic Review and Meta-Analysis

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The results of prospective cohort studies regarding the role of salt intake and subsequent gastric cancer risk are inconsistent. Thus, we performed a systematic review and meta-analysis to summarize the strength of the association of salt intake with gastric cancer morbidity and mortality. PubMed, EmBase, and Cochrane Library were systematically searched to identify eligible studies published throughout September 2021. The effect estimates with 95% confidence intervals (CIs) for gastric cancer morbidity or mortality in each study were applied to calculate the pooled results; these analyses were performed using the random-effects model. Twenty-six prospective cohort studies involving 4,956,350 individuals were selected; these studies reported 19,301 cases of gastric cancer and 2,871 cases of gastric cancer-associated mortality. High (RR: 1.25; 95%CI: 1.10–1.41; $P = 0.001$) or moderate (RR: 1.20; 95%CI: 1.04–1.38; $P = 0.012$) salt intake was associated with a greater risk of gastric cancer. High pickled food intake was associated with an increased gastric cancer risk (RR: 1.28; 95%CI: 1.05–1.57; $P = 0.017$), while moderate pickled foods intake had no significant effect on gastric cancer risk (RR: 1.10; 95%CI: 0.88–1.37; $P = 0.390$). Neither high (RR: 1.14; 95%CI: 0.95–1.36; $P = 0.161$) nor moderate (RR: 1.10; 95%CI: 0.87–1.40; $P = 0.436$) salted fish intake were associated with gastric cancer risk. A high intake of processed meat was significantly associated with a higher risk of gastric cancer (RR: 1.24; 95%CI: 1.03–1.49; $P = 0.023$), while moderate processed meat intake had no significant effect on the gastric cancer risk (RR: 1.01; 95%CI: 0.92–1.11; $P = 0.844$). High (RR: 1.04; 95%CI: 0.90–1.19; $P = 0.626$) and moderate (RR: 1.02; 95%CI: 0.94–1.11; $P = 0.594$) miso-soup intake had no effects on the gastric cancer risk. High intakes of salt, pickled food, and processed meat are associated with significantly increased risks of gastric cancer; these increased risks are also seen when participants consumed moderate amounts of salt.

Keywords: gastric cancer, salt intake, risks, meta-analysis, systematic review

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INTRODUCTION

Gastric cancer is the fifth most common type of cancer and is the third leading cause of cancer-related deaths worldwide (1). There were more than one million new cases of gastric cancer diagnosed in 2018, and the number of gastric cancer-related deaths reached 783,000 (1). Nearly 70% of new gastric cancer cases occurred in developing countries, especially, in China.

Therefore, additional potential risk factors for this condition should be identified for preventing its progression. Studies have already found several lifestyle-associated factors could prevent the risk of gastric cancer, including the intake of citrus fruits (2), flavonols (3), dietary nitrates, nitrites, nitrosamines (4), a Mediterranean diet (5), dairy products (6), vitamin A, vitamin C, vitamin E (7), cruciferous vegetables (8), and dietary fiber (9), and physical activity (10). Moreover, several potential risk factors for gastric cancer, including intake of coffee, dietary fat, red meat, obesity, and smoking, have been identified (11–15). However, other dietary factors should be identified to further prevent the risk of gastric cancer.

Previous study have already demonstrated that increased dietary sodium intake is a modifiable risk factor for health (16). They point out reduced sodium intake significantly reduced blood pressure without any significant effects on blood lipids, catecholamine levels, and renal function for non-acutely ill adults. Moreover, reduced sodium intake was associated with a reduced risk of stroke and fatal coronary heart disease in adults. The World Health Organization currently recommends a salt intake of <2 g/d, a level that is largely based on a relatively small and short-term clinical trials evaluating the effects of moderate salt intake in the general population (17). Several systematic review and meta-analyses have illustrated the association of salt intake with the risk of gastric cancer (18–21). Excessive salt intake plays a dual effect at the initial stages, including gastritis and atrophy. Moreover, it might play an important role on the later stages of carcinogenesis through intestinal metaplasia and dysplasia stages (22). However, whether the strength of this association differs according to various characteristics in individuals remains unclear. Clarifying the optimal salt intake in the general population for preventing gastric cancer is particularly important, as this has not yet been definitively determined. Therefore, in the present study, we performed a systematic review and meta-analysis of prospective cohort studies to assess the strength of the association of dietary salt intake with the risk of gastric cancer; further, the comparison of this association in individuals with various characteristics was performed.

METHODS

Data Sources, Search Strategy, and Selection Criteria

The Meta-analysis of Observational Studies in Epidemiology guidelines were applied to perform and report this systematic review and meta-analysis (23). Studies designed as prospective cohort studies and those that assessed the association of dietary salt intake with the risk of gastric cancer were eligible for inclusion in our study, and the publication language was restricted to English. PubMed, EmBase, and Cochrane Library were searched for articles published throughout September 2021, using ("Salt" OR "Salty" OR "Salted" OR "Sodium" OR "Diet" OR "Dietary" OR "Food" OR "Snack" OR "Bread" OR "Miso" OR "Pickle" OR "Processed fish" OR "Processed meat" OR "Salty fish)" AND ("Stomach cancer" OR "Gastric cancer)" AND "prospective" AND "human" AND "English" as the search terms. The reference lists of relevant original articles were also manually reviewed to identify any new eligible studies.

The literature search and study selection were independently undertaken by two authors, and any disagreements between these two authors were settled by mutual discussion until a consensus was reached. The inclusion criteria were as follows: (1) Study design: the study had to have a prospective cohort design; (2) Exposure: total dietary salt intake, pickled food, salted fish, processed meat, and miso-soup; (3) Control: the lowest intake of salt or a specific food category; (4) Outcome: gastric cancer-associated morbidity or mortality; and (5) Participants: general population or individuals without gastric cancer at inclusion. Retrospective cohort, traditional case-control, and series studies were excluded because the results of these studies may be susceptible to biases resulting from various confounding factors.

Data Collection and Quality Assessment

Two authors (DY and SY) independently performed the data extraction and quality assessment, and any conflicts between these authors were examined and adjudicated by an additional author (GZ) by referring to the original studies. The collected characteristics and data included the study group or first author's name, publication year, country, sample size, age of participants, numbers of men and women, number of cases showing gastric cancer-associated morbidity or mortality, number of dietary questionnaire items, follow-up duration, reported effect estimates and 95% CI values, and covariates in the fully adjusted model. For studies that reported several multivariable adjusted effect estimates, the effect estimate that was maximally adjusted for potential confounders was used. The Newcastle-Ottawa Scale (NOS) was utilized to assess the methodological quality, which is quite comprehensive and has been partially validated for evaluating the quality of observational studies in meta-analyses (24). The NOS, based on selection [4 items (four stars): representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of salt consumption, and demonstration that outcomes was not present at start of study], comparability [one item (two stars): comparability on the basis of the design or analysis], and outcome [three items (three stars): assessment of outcome, adequate follow-up duration, and adequate follow-up rate], and the "star system," ranged from 0 to 9 for each study.

Statistical Analysis

The association of the intake of salt or specific foods (pickled food, salted fish, processed meat, and miso-soup) with the risk of gastric cancer-associated morbidity or mortality was analyzed based on the effect estimate [risk ratio (RR), hazard ratio (HR), or odds ratio (OR)] and its 95% CI published in each study. The categories for salt or specific foods were divided based on tertiles, and the random-effects model was utilized to calculate the pooled RRs and 95% CIs for high or moderate vs. low salt or specific food intake (25). The I^2 and Cochren Q statistic were used to assess heterogeneity across the included studies; significant heterogeneity was defined at $I^2 > 50.0\%$ or $P < 0.10$ (26, 27). The stability of the pooled conclusions was assessed

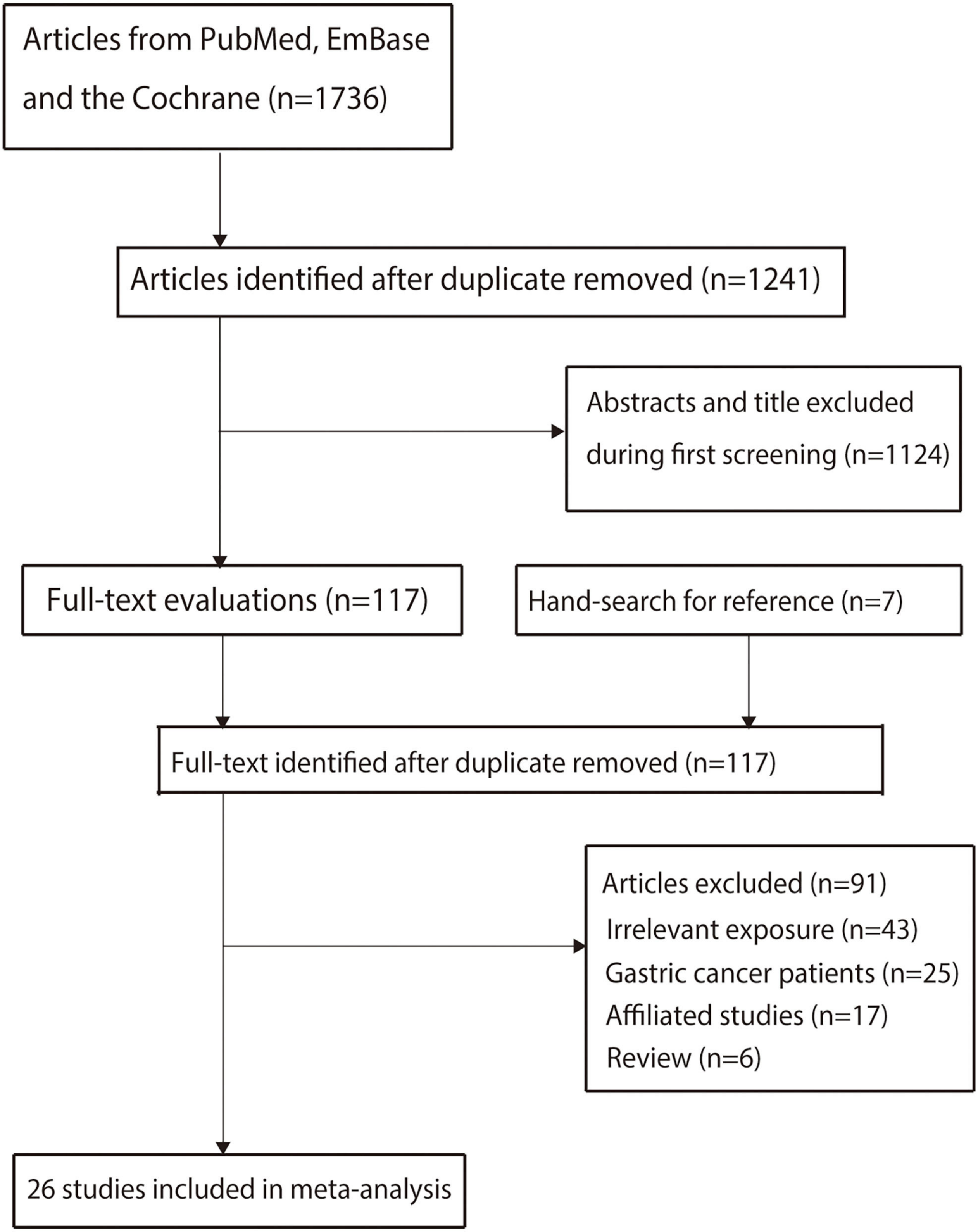

FIGURE 1 | PRISMA flowchart of the literature search and study selection process.

TABLE 1 | Baseline characteristic of studies included in meta-analysis.

Study	Country	Sample size	Age (year)	Gender (men/women)	No of GC cases	Salt questionnaire	Follow-up (year)	Adjusted factors	NOS score
JHCS 1990 (32)	Japan	7,990	> 45.0	7,990/0	Incidence (150)	17 items	17.5	Age	7
Kneller 1991 (33)	Norway	17,633	>35.0	17,633/0	Mortality (75)	35 items	20.0	Years of birth and smoking	7
Kato 1992 (34)	Japan	3,914	> 45.0	1,851/2,063	Incidence (45)	10 items	4.4	Age, sex, and residence	6
Kato 1992 (35)	Japan	9,753	> 30.0	NA	Mortality (57)	25 items	5.7	Age, and sex	6
HHSP 1998 (36)	US	11,907	46.4	5,610/6,297	Incidence (108)	13 items	14.8	Age, education, Japanese place of birth (for men added smoking and alcohol)	8
Knekt 1999 (37)	Finland	9,985	>15.0	5,274/4,711	Incidence (68)	NA	24.0	Sex, age, municipality, smoking and TE	7
CPS II 2001 (38)	US	970,045	56.0	436,654/533,391	Mortality (1,349)	32 items	14.0	Age, education, smoking, BMI, multivitamin and vitamin C use, aspirin use, race, and family history	8
Ngoan 2002 (39)	Japan	13,250	52.7	5,917/7,333	Mortality (116)	254 items	8.8	Age, gender, smoking, processed meat, liver, cooking or salad oil, suimono and pickled fruit	7
TNCS 2003 (40)	Netherlands	120,852	55.0–69.0	58,279/62,573	Incidence (282)	150 items	6.3	Age, gender, smoking, education, family history of stomach disorders and GC	8
Khan 2004 (41)	Japan	3,158	>40.0	1,524/1,634	Mortality (51)	37 items	14.3	Age, and smoking	6
CGCS group 2004 (42)	China	1,630	42.2	880/750	Incidence (18)	NA	7.5	Active treatment	8
JACC 2005 (43)	Japan	110,792	40.0–79.0	NA	Mortality (859)	33 items	12.0	Age	7
LSS 2005 (44)	Japan	38,576	34.0–98.0	14,885/23,691	Incidence (1,280)	22-items	20.0	Sex, sex-specific age, city, radiation dose, smoking, and education level	7
LGPT 2005 (45)	China	29,584	40.0–69.0	13,313/16,271	Incidence (1,452)	9 items	15.0	Age, gender, or smoking	8
Kurosawa 2006 (46)	Japan	8,035	> 30.0	3,652/4,383	Mortality (76)	29 items	11.0	Age, gender	7
THS 2006 (47)	Japan	2,467	57.9	1,023/1,444	Incidence (93)	70 items	14.0	Age, gender, *H pylori* infection, atrophic gastritis, history of peptic ulcer, family history of cancer, BMI, DM, TC, PA, alcohol, smoking and dietary factors (TE, TP, carbohydrate, B1-B2-C vitamin and dietary fiber)	7
SMC 2006 (48)	Sweden	61,433	53.4	0/61,433	Incidence (156)	67 items	18.0	Age, education, BMI, TE, alcohol, fruits, and vegetables	8
EPIC 2006 (49)	Europe	521,457	51.7	153,447/368,010	Incidence (330)	88–266 items	6.5	Sex, height, weight, education level, smoking, work and leisure PA, alcohol, TE, vegetable, citrus fruit, non-citrus fruit intake, red meat, and poultry	8
Sjodahl 2008 (50)	Norway	73,133	49.0	35,955/37,178	Incidence (313)	NA	15.4	Age, smoking, alcohol, PA and occupation	6
Kim 2010 (51)	Korea	2,248,129	30.0–80.0	1,420,981/827,148	Incidence (12,393)	13 items	7.0	Age, sex, BMI, smoking, alcohol, PA, and family history of cancer	7
JPHC 2010 (52)	Japan	77,500	45.0–74.0	35,730/41,770	Incidence (867)	138 items	7.7	Sex, age, BMI, smoking, alcohol, PA, and quintiles of energy, potassium, and calcium	8
Murata 2010 (53)	Japan	6,830	50.8	3,074/3,756	Mortality (87)	NA	13.9	Age, BMI, PA, smoking, alcohol, DM, vegetable, fruit, tea, red meat and processed meat	8
NIH-AARP 2011 (54)	US	337,074	50.0–71.0	177,792/159,282	Incidence (955)	124 item	10.0	Age, sex, BMI, education, ethnicity, smoking, alcohol, PA, and the daily intake of fruit, vegetables, saturated fat	8

(Continued)

TABLE 1 | Continued

Study	Country	Sample size	Age (year)	Gender (men/women)	No of GC cases	Salt questionnaire	Follow-up (year)	Adjusted factors	NOS score
SCHS 2017 (55)	Singapore	63,257	45.0–74.0	29,220/34,037	Incidence (691)	165 items	16.9	Age, interview year, father's dialect, gender, and education	8
Thapa 2019 (56)	US	260	43.8	88/172	Incidence (10)	NA	11.0–12.0	Age, sex, car ownership, and fruit and vegetable intake	6
KoGES and KMCC 2020 (57)	Korea	196,384/11,322	> 40.0 />20.0	NA	Mortality (201)/ Incidence (90)	103 items	7.4/13.3	Age, sex, survey year, BMI, smoking, and alcohol	7

GC, gastric cancer; BMI, body mass index; DM, diabetes mellitus; TC, total cholesterol; PA, physical activity; TE, total energy; TP, total protein.

using sensitivity analyses through the sequential removal of each individual study (28). Stratified analyses were performed for high or moderate salt or specific foods on gastric cancer risk according to the country, gender, reported outcomes, follow-up duration, and adjustment for educational level, body mass index (BMI), alcohol, smoking, or physical activity (PA); further, the ratio between subgroups were compared based on the RRs and 95%CIs in each subset (29). Publication biases were assessed using both qualitative and quantitative methods, including funnel plots, and the Egger and Begg tests (30, 31). The inspection levels were two-sided for pooled results, and differences with $P < 0.05$ were regarded statistically significant. The STATA software was used to perform all the statistical analyses in this study (version 10.0; Stata Corporation, College Station, TX, USA).

RESULTS

Literature Search

A total of 1,736 articles were identified in electronic searches, and 1,241 studies were retained after duplicate articles were removed. Further, 1,124 studies were excluded because these studies contained irrelevant titles and abstracts. The remaining 117 studies were examined for further full-text evaluations, and 91 studies were excluded because: they contained irrelevant exposure ($n = 43$), they contained pre-existing gastric cancer patients ($n = 25$), they were affiliate studies ($n = 17$), and they were reviews ($n = 6$). A review of the reference lists of the relevant studies did not find any new eligible study. Finally, 26 prospective cohort studies were selected for the final meta-analysis (32–57); the flowchart representing the study selection process is shown in **Figure 1**.

Study Characteristics

The baseline characteristics of the studies and participants are summarized in **Table 1**. A total of 4,956,350 individuals were recruited from 26 studies, and 19,301 cases of gastric cancer and 2,871 cases of gastric cancer-associated mortality were reported. The follow-up duration for each study ranged from 4.4 to 24.0 years, and 260–2,248,129 individuals were included in each study. Sixteen studies were performed in Asia, six studies were performed in Europe, and the remaining four studies were performed in the US. Eighteen studies reported the association of the intake of salt or specific foods with the risk of gastric cancer incidence, and nine studies reported the association of the intake of salt or specific foods with the risk of gastric cancer-associated mortality. Eleven studies showed an NOS score of eight stars, 10 studies showed an NOS score of seven stars, and the remaining five studies showed an NOS score of six stars.

Salt Intake and Gastric Cancer Risk

The numbers of studies reporting the risk of gastric cancer with regard to high and moderate salt intake were 13 and 10, respectively. We noted that high (RR: 1.25; 95%CI: 1.10–1.41; $P = 0.001$) or moderate (RR: 1.20; 95%CI: 1.04–1.38; $P = 0.012$) salt intake were associated with a greater risk of gastric cancer (**Figure 2**).

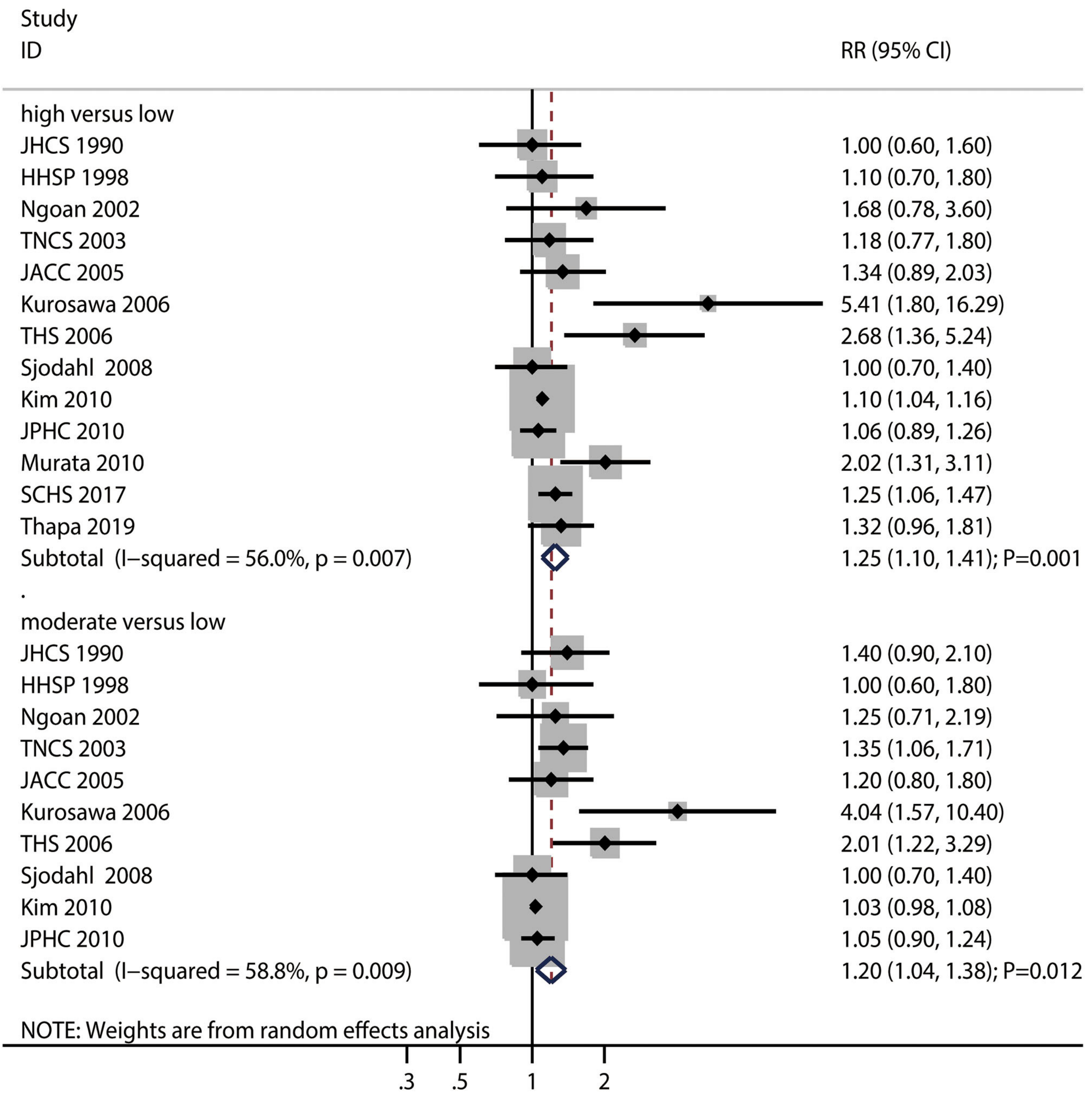

FIGURE 2 | Association between high or moderate salt intake and subsequent gastric cancer risk.

There was significant heterogeneity for high ($I^2 = 56.0\%$; $P = 0.007$) and moderate ($I^2 = 58.8\%$; $P = 0.009$) salt intake among the included studies. Sensitivity analyses indicating the pooled conclusions for gastric cancer risk with regard to high and moderate salt intake are robust and not affected by any specific study (**Supplemental 1**). Subgroup analysis found that the gastric cancer risk related to high salt intake increased significantly in most subgroups, while high salt intake was not associated with the risk of gastric cancer if the pooled studies were performed in US or Europe and included female individuals. In case of the gastric cancer risk related to high salt intake, the gastric cancer incidence was lower than the gastric cancer mortality; the association between gastric cancer risk and high salt intake after $\geq$ 10.0 years of follow-up was greater than that observed after $<$ 10.0 years of follow-up (**Table 2**). In addition, the subgroup analysis indicated that moderate salt intake was associated with an increased risk of gastric cancer in case of pooled trials performed in Asia, studies reporting gastric cancer incidence, and studies involving a follow-up duration of $\geq$ 10.0 years, irrespective of the educational level status and adjustment

TABLE 2 | Subgroup analysis for high vs. low salt intake and the risk of gastric cancer.

Group	RR and 95%CI	*P*-value	Heterogeneity (%)	*P*-value for heterogeneity	Ratio between subgroups	*P*-value for interaction test
Country						
US or Europe	1.16 (0.96–1.40)	0.130	0.0	0.706	0.88 (0.68–1.13)	0.315
Asia	1.32 (1.11–1.55)	0.001	69.0	0.001		
Gender						
Men	1.10 (1.03–1.17)	0.002	0.0	0.812	1.01 (0.88–1.16)	0.898
Women	1.09 (0.96–1.23)	0.171	0.0	0.750		
Outcomes						
GC incidence	1.14 (1.05–1.25)	0.003	25.0	0.222	0.60 (0.39–0.93)	0.022
GC mortality	1.89 (1.24–2.89)	0.003	50.6	0.108		
Follow-up duration (years)						
≥ 10.0	1.38 (1.12–1.69)	0.002	57.6	0.015	1.25 (1.01–1.55)	0.037
< 10.0	1.10 (1.04–1.16)	< 0.001	0.0	0.692		
Adjusted educational						
Yes	1.23 (1.06–1.42)	0.006	0.0	0.866	0.95 (0.76–1.18)	0.626
No	1.30 (1.10–1.54)	0.003	65.0	0.002		
Adjusted BMI						
Yes	1.32 (1.03–1.69)	0.026	79.0	0.003	1.07 (0.81–1.43)	0.630
No	1.23 (1.07–1.43)	0.004	22.2	0.246		
Adjusted alcohol						
Yes	1.21 (1.01–1.46)	0.036	65.9	0.012	0.93 (0.72–1.20)	0.581
No	1.30 (1.09–1.55)	0.003	28.1	0.214		
Adjusted smoking						
Yes	1.22 (1.04–1.42)	0.015	55.9	0.026	0.92 (0.70–1.22)	0.583
No	1.32 (1.04–1.66)	0.020	48.2	0.103		
Adjusted PA						
Yes	1.24 (1.01–1.52)	0.038	72.7	0.005	0.98 (0.75–1.26)	0.856
No	1.27 (1.09–1.49)	0.002	19.6	0.275		

RR, relative risk; CI, confidence interval; GC, gastric cancer; BMI, body mass index; PA, physical activity.

for BMI, alcohol intake, and PA. Moreover, the strength of the association of gastric cancer risk and moderate salt intake was lower in studies with adjustment for alcohol intake than in studies without adjustment for alcohol intake (**Table 3**).

Pickled Food Intake and Gastric Cancer Risk

The numbers of studies reporting the risk of gastric cancer related to high and moderate pickled food intake were 12 and nine, respectively. We noted that high pickled food intake was associated with an increased risk of gastric cancer (RR: 1.28; 95%CI: 1.05–1.57; $P = 0.017$), while moderate pickled food intake was not (RR: 1.10; 95%CI: 0.88–1.37; $P = 0.390$) (**Figure 3**).

Moreover, there was significant heterogeneity for gastric cancer risk related to high ($I^2 = 79.4\%$; $P < 0.001$) and moderate ($I^2 = 79.7\%$; $P < 0.001$) pickled food intake across the included studies. Sensitivity analyses found that the pooled conclusions for gastric cancer related to high and moderate pickled food intake were stable after the sequential exclusion of individual studies (**Supplemental 1**). The subgroup analysis demonstrated that high pickled food intake was associated with an increased risk of gastric cancer in case of pooled studies performed in Asia, studies with a follow-up of ≥ 10.0 years, and studies without adjustment for the educational level, BMI, alcohol intake, and PA (**Supplemental 2**). Moreover, the results of the subgroup analyses for moderate pickled food intake and gastric cancer risk were consistent with those of the overall analysis and remained statistically non-significant (**Supplemental 2**).

Salted Fish Intake and Gastric Cancer Risk

The numbers of studies reporting the risk of gastric cancer related to high and moderate salted fish intake were 11 and eight, respectively. We noted that high (RR: 1.14; 95%CI: 0.95–1.36; $P = 0.161$) or moderate (RR: 1.10; 95%CI: 0.87–1.40; $P = 0.436$) salted fish intakes were not associated with the risk of gastric cancer (**Figure 4**), and a potential significant heterogeneity for gastric cancer risk related to high ($I^2 = 49.7\%$; $P = 0.030$) and moderate ($I^2 = 73.7\%$; $P < 0.001$) salted fish intake was noted among the included studies.

The pooled conclusions for gastric cancer risks related to high and moderate salted fish intake were robust and not affected by the exclusion of any particular study (**Supplemental 1**). The subgroup analysis found that high salted fish intake was associated with an increased risk of gastric cancer in case of

TABLE 3 | Subgroup analysis for moderate vs. low salt intake and the risk of gastric cancer.

Group	RR and 95%CI	*P*-value	Heterogeneity (%)	*P*-value for heterogeneity	Ratio between subgroups	*P*-value for interaction test
Country						
US or Europe	1.18 (0.95–1.46)	0.132	17.3	0.299	0.95 (0.72–1.26)	0.731
Asia	1.24 (1.03–1.49)	0.022	65.8	0.007		
Gender						
Men	1.04 (0.98–1.10)	0.217	0.0	0.812	1.04 (0.94–1.15)	0.437
Women	1.00 (0.92–1.08)	0.981	0.0	0.521		
Outcomes						
GC incidence	1.15 (1.00–1.31)	0.045	54.9	0.038	0.72 (0.40–1.31)	0.285
GC mortality	1.59 (0.89–2.83)	0.115	63.9	0.063		
Follow-up duration (years)						
≥ 10.0	1.38 (1.02–1.87)	0.036	57.8	0.037	1.27 (0.92–1.75)	0.152
< 10.0	1.09 (0.97–1.21)	0.134	41.5	0.162		
Adjusted educational						
Yes	1.29 (1.03–1.60)	0.024	0.0	0.326	1.08 (0.82–1.43)	0.564
No	1.19 (1.01–1.40)	0.032	60.2	0.014		
Adjusted BMI						
Yes	1.12 (0.93–1.34)	0.242	71.2	0.031	0.88 (0.67–1.15)	0.358
No	1.27 (1.04–1.54)	0.018	32.1	0.183		
Adjusted alcohol						
Yes	1.07 (0.94–1.22)	0.283	42.8	0.136	0.77 (0.59–1.00)	0.048
No	1.39 (1.11–1.74)	0.004	28.1	0.234		
Adjusted smoking						
Yes	1.13 (0.99–1.28)	0.066	49.6	0.064	0.70 (0.42–1.18)	0.183
No	1.61 (0.97–2.66)	0.066	62.7	0.068		
Adjusted PA						
Yes	1.08 (0.94–1.26)	0.276	57.0	0.073	0.81 (0.62–1.04)	0.101
No	1.34 (1.08–1.65)	0.007	25.4	0.243		

RR, relative risk; CI, confidence interval; GC, gastric cancer; BMI, body mass index; PA, physical activity.

pooled studies reporting gastric cancer incidence and studies with adjustment for the PA; moreover, the strength of the association of gastric cancer risk with high salted fish intake in studies with the adjustment for PA was higher than that in case of studies without the adjustment for adjusted PA (**Supplemental 2**). Moreover, the subgroup analysis found that moderate salted fish intake was associated with an increased risk of gastric cancer if the follow-up duration was < 10.0 years, and in case for studies with the adjustment for PA, while moderate salted fish intake was associated with a reduced risk of gastric cancer in women. The differences between the subgroups in the analyses based on gender, follow-up duration, and adjustment for PA were statistically significant (**Supplemental 2**).

Processed Meat Intake and Gastric Cancer Risk

The numbers of studies reporting the risk of gastric cancer related to high and moderate processed meat intake were eight and six, respectively. We noted that high processed meat intake was associated with an increased risk of gastric cancer (RR: 1.24; 95%CI: 1.03–1.49; $P = 0.023$), while moderate processed meat intake had no significant effect on the risk of gastric cancer (RR: 1.01; 95%CI: 0.92–1.11; $P = 0.844$) (**Figure 5**).

There was significant heterogeneity for gastric cancer risk related to high processed meat intake ($I^2 = 63.4\%$; $P = 0.008$), while no evidence of heterogeneity for gastric cancer risk related to moderate processed meat intake ($I^2 = 0.0\%$; $P = 0.461$) was noted. The pooled conclusions for gastric cancer risk related to high processed meat intake were variable, while the gastric cancer risk related to moderate processed meat intake was stable (**Supplemental 1**). The subgroup analyses showed that high processed meat intake was associated with an increased risk of gastric cancer in case of pooled studies performed in the US or Europe, studies with a follow-up duration of < 10.0 years, studies with adjustment for educational level, and studies without adjustment for smoking and PA (**Supplemental 2**). Moreover, moderate processed meat intake was not associated with the risk of gastric cancer in all subgroups (**Supplemental 2**).

Miso-Soup Intake and Gastric Cancer Risk

The numbers of studies reporting the risk of gastric cancer with regard to high and moderate miso-soup intake were nine and seven, respectively. We noted that high (RR: 1.04; 95%CI:

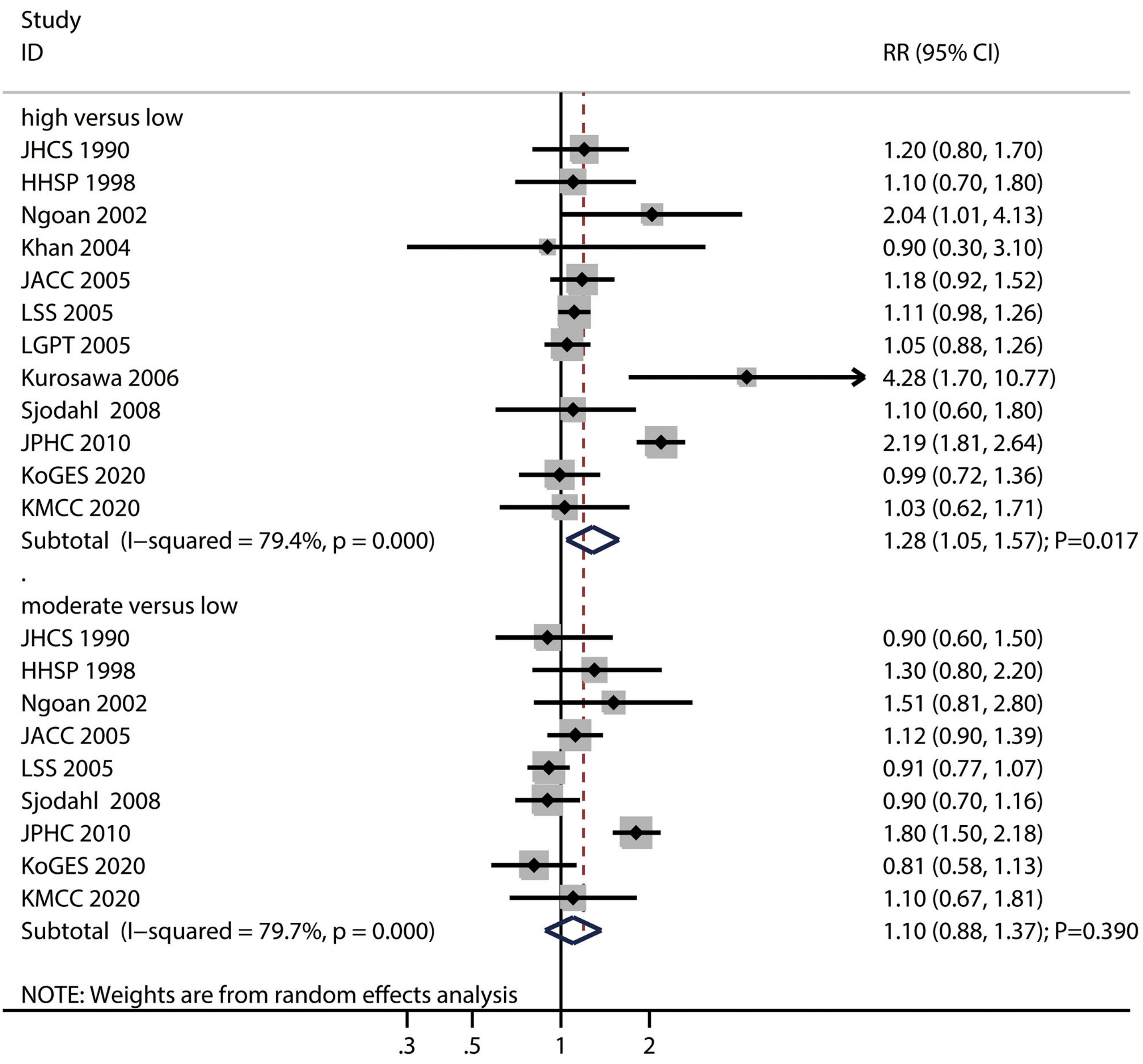

FIGURE 3 | Association between high or moderate pickled food intake and subsequent gastric cancer risk.

0.90–1.19; $P = 0.626$) and moderate (RR: 1.02; 95%CI: 0.94–1.11; $P = 0.594$) miso-soup intake were not associated with the risk of gastric cancer, and no significant heterogeneity for gastric cancer related to high ($I^2 = 38.8\%$; $P = 0.109$) and moderate ($I^2 = 0.0\%$; $P = 0.993$) miso-soup intake was observed (**Figure 6**). The pooled conclusions for gastric cancer risk related to high and moderate miso-soup intakes were found to be robust after the sequential removal of single studies (**Supplemental 1**). The results of the subgroup analyses showed that the gastric cancer risks related to high and moderate miso-soup intakes were consistent with the findings of the overall analysis in all subgroups (**Supplemental 2**).

Publication Bias

Review of the funnel plots could not rule out the potential of publication bias for conclusions regarding high and moderate intake of salt or specific foods (**Supplemental 3**). We noted potential significant publication bias for gastric cancer risk related to high and moderate salt intake, but no significant publication biases for gastric cancer risk related to high and moderate pickled food, salted fish, processed meat, and miso-soup intakes. The conclusions remained unchanged after adjustment for publication bias for gastric cancer related to high and moderate salt intake using the trim and fill method (58).

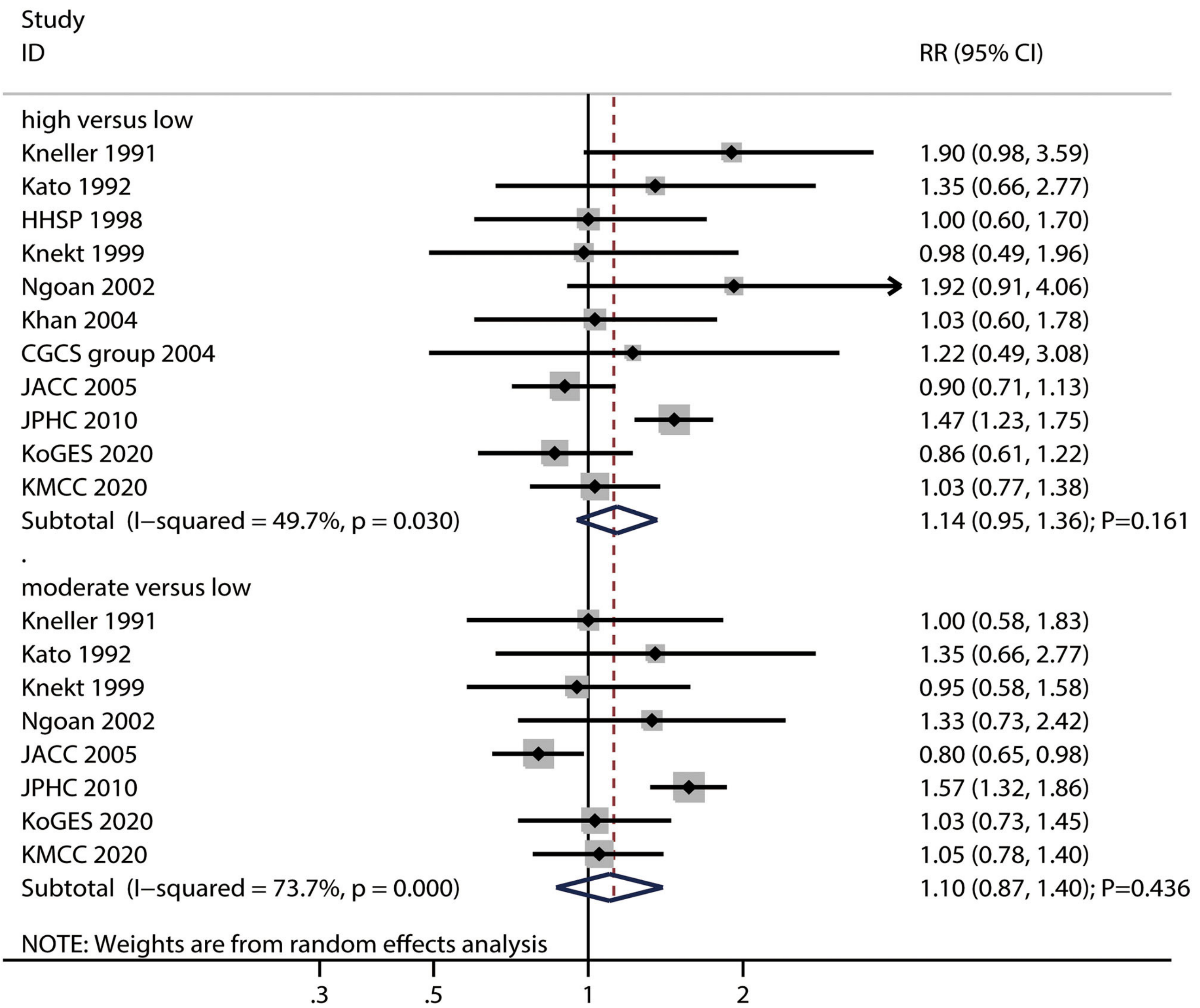

FIGURE 4 | Association between high or moderate salted fish intake and subsequent gastric cancer risk.

DISCUSSION

Our study intended to assess the association of the intake of salt or specific foods with the risk of gastric cancer based on high-quality prospective cohort studies. A total of 4,956,350 individuals with 19,301 cases of gastric cancer and 2,871 cases of gastric cancer-associated mortality from 26 studies were identified and a broad range of characteristics of the studies or individuals were considered. The findings of this study found that high and moderate salt intakes increase the risk of gastric cancer. Moreover, high pickled food and processed meat intakes were associated with an increased risk of gastric cancer, while moderate pickled food and processed meat intakes were not. Furthermore, salted fish and miso-soup intakes were not associated with the risk of gastric cancer, irrespective of whether the intakes were high or moderate. The associations of salt or specific food intake with the risk of gastric cancer were affected by gender, reported outcomes, follow-up duration, and adjustment for alcohol intake and PA. Finally, considering the satisfactory quality of the included studies, the findings of this study are recommendable for the general population.

Several systematic review and meta-analyses have already addressed the potential role of dietary salt or specific foods in increasing the risk of gastric cancer (19, 21). A study conducted by D'Elia et al. found that high and moderate dietary salt intakes were associated with an increased risk of gastric cancer, and this association was stronger in case of Japanese populations and a higher consumption of selected salt-rich foods (19). Similarly, Ge et al. identified 11 studies and found that dietary salt intake was positively related to the risk of gastric cancer (21). However, stratified analyses performed on the basis of gender and adjustment for different parameters levels were not considered. Therefore, we performed this study to systematically assess the

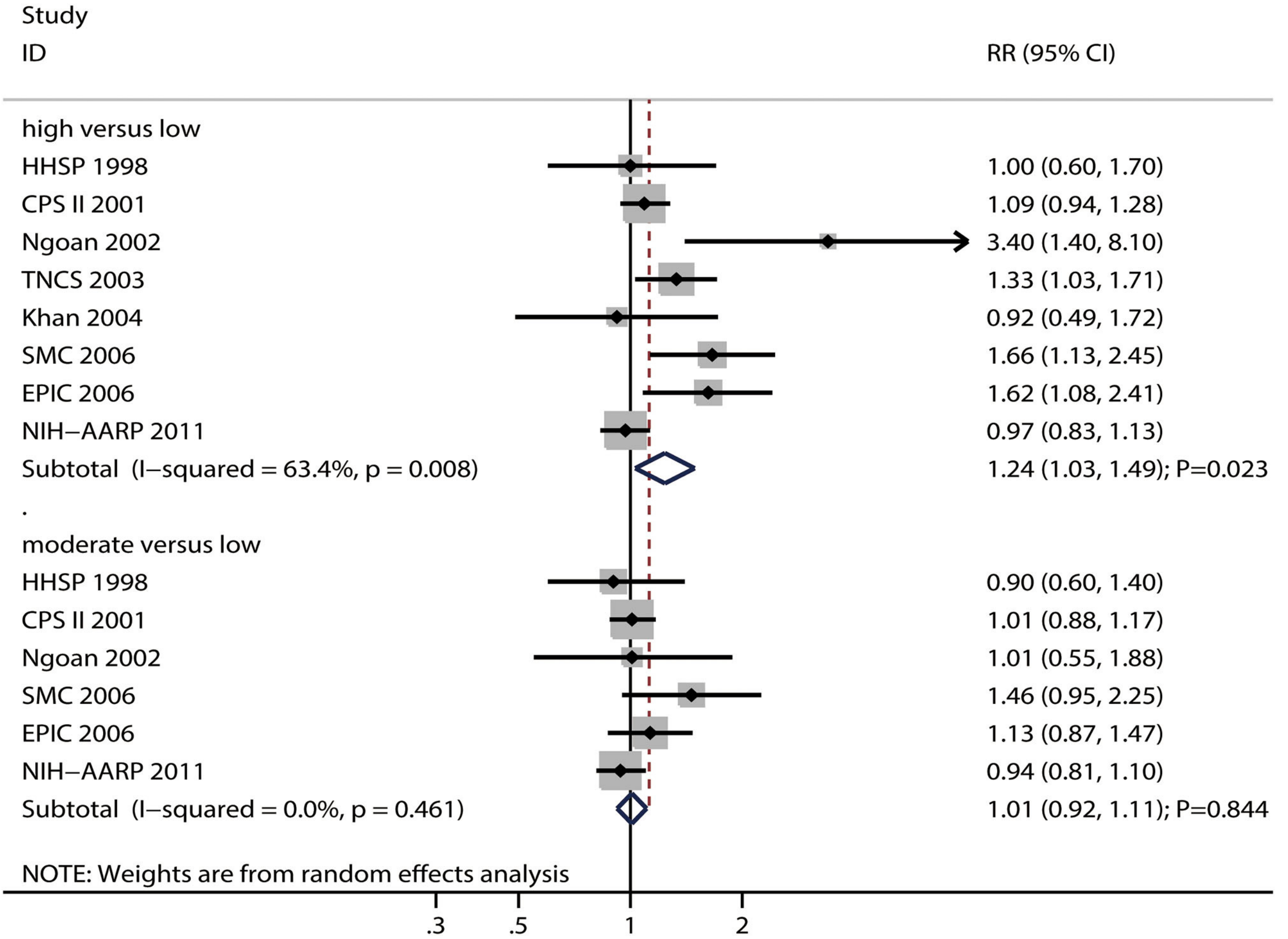

FIGURE 5 | Association between high or moderate processed meat intake and subsequent gastric cancer risk.

associations of salt or specific food intakes with the risk of gastric cancer.

Our study found that high or moderate salt intakes were associated with an increased risk of gastric cancer, which are consistent with the findings of previous meta-analyses (19, 21). Several potential mechanisms could explain the increase in the gastric cancer risk associated with the high intakes of salt, pickled food, and processed meat: (1) dietary salt was associated with N-methyl-N-nitro-N-nitrosoguanidine, which could induce carcinogenic effects in the stomach (59); (2) the mucosal barrier could be destroyed by high salt concentrations in the intragastric region, which may cause inflammation and damage, and subsequently, diffuse erosion and degeneration of the gastric mucosa. These symptoms could induce proliferous changes and enhance the effects of food-derived carcinogens (60); and (3) the mucosal damage could enhance *H. pylori* colonization in mice and humans, leading to chronic gastritis, which is associated with a greater risk of gastric cancer (61–63).

We noted that high pickled food and processed meat intakes increased the risk of gastric cancer, while moderate pickled food and processed meat intake did not affect the risk of gastric cancer. Further, increased intakes of salted fish and miso-soup did not affect the risk of gastric cancer. Several reasons could explain these results: (1) the follow-up duration for these studies were shorter than the duration needed to show a clinical benefit, resulting in broad confidence intervals and no statistically significant associations; (2) the items of food-frequency questionnaire across the included studies differed, which may introduce biases with regard to the association of the intake of salt or specific foods with the risk of gastric cancer; (3) the net effect estimates could be affected by the levels of salt or specific foods in the control arm; (4) the adjusted factors across the included studies are different, which may introduce biases with regard to the pooled results; and (5) the study quality and number of studies reported for each exposure are different, and thus, the robustness of pooled conclusions could be affected.

The subgroup analyses found that the potential associations of the intakes of salt or specific foods with the risk of gastric cancer could affected by gender, reported outcomes, follow-up duration, and adjustment for alcohol intake and PA. The potential reasons

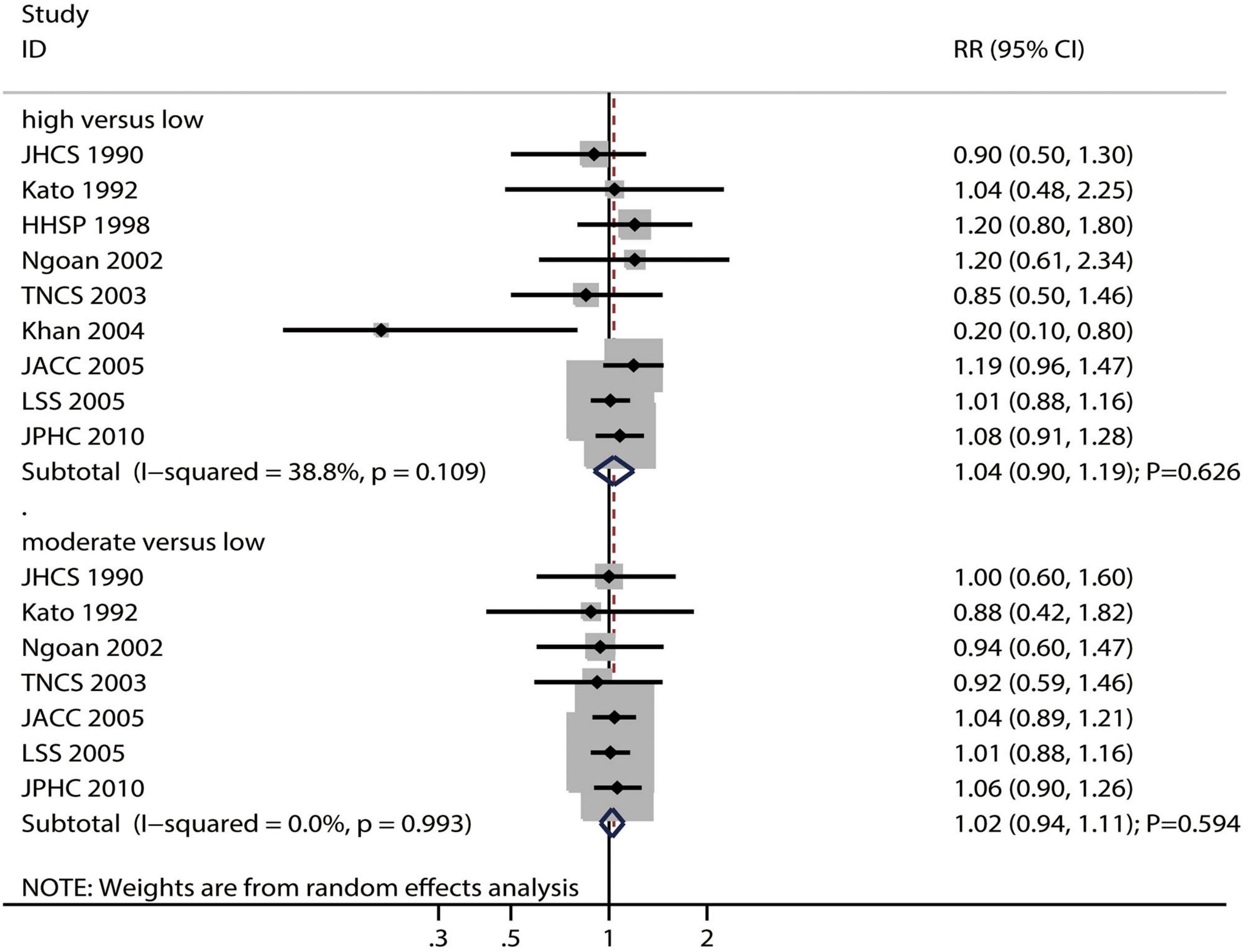

FIGURE 6 | Association between high or moderate miso-soup intake and subsequent gastric cancer risk.

for these differences are: (1) gender, reported outcomes, and follow-up duration could affect gastric cancer incidence and gastric cancer-associated mortality, and the power to detect potential associations are different; and (2) alcohol intake and PA are significantly associated with the risk of gastric cancer; thus, complete adjustment for both these parameters should be performed to avoid potential confounding bias. Moreover, we noted that the associations of the intake of salt or specific foods with the risk of gastric cancer differed in various countries. The potential reason for this could be that Asia shows the highest incidence of gastric cancer in the world, i.e., over 4–7 times higher than that in the US or Europe; this could make it easier to detect the differences in this relationship in different regions (1).

Although our analysis is based on prospective cohort studies, several limitations of the present study should be acknowledged. First, the levels of adjustment for various parameters across the included studies differed; because these factors play an important role in the progression of gastric cancer, their adjustments must be consistent. Second, the differences in the food-frequency questionnaire could affect the level of exposure to each food type, which might introduce biases in the relationship between the intakes of dietary salt or specific foods and gastric cancer risk. Third, the dose-response analysis was restricted owing to the unavailability of cases and people or person–year data in each category. Fourth, there are inherent limitations associated with the analysis based on published articles, including inevitable publication bias and restricted detailed analyses.

CONCLUSION

In summary, the results of the present study suggest that dietary salt intake may have harmful effects on the risk of gastric cancer in terms of gastric cancer-associated morbidity and mortality. Moreover, high pickled food and processed meat intakes were associated with an increased risk of gastric cancer. Further randomized controlled trials should be performed to assess the effects of reduced dietary salt intake on the risk of gastric cancer according to different characteristics of the subjects.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GZ: conception and design, administrative support, and provision of study materials or patients. DY: collection and assembly of data. SY and BW: data analysis and interpretation. GZ, DY, SY, and BW: manuscript writing and final approval of manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.801228/full#supplementary-material

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* (2018) 68:394–424. doi: 10.3322/caac.21492
2. Bertuccio P, Alicandro G, Rota M, Pelucchi C, Bonzi R, Galeone C, et al. Citrus fruit intake and gastric cancer: the stomach cancer pooling (StoP) project consortium. *Int J Cancer.* (2019) 144:2936–44. doi: 10.1002/ijc.32046
3. Xie Y, Huang S, Su Y. Dietary flavonols intake and risk of esophageal and gastric cancer: a meta-analysis of epidemiological studies. *Nutrients.* (2016) 8:91. doi: 10.3390/nu8020091
4. Song P, Wu L, Guan W. Dietary nitrates, nitrites, and nitrosamines intake and the risk of gastric cancer: a meta-analysis. *Nutrients.* (2015) 7:9872–95. doi: 10.3390/nu7125505
5. Schwingshackl L, Hoffmann G. Adherence to mediterranean diet and risk of cancer: an updated systematic review and meta-analysis of observational studies. *Cancer Med.* (2015) 4:1933–47. doi: 10.1002/cam4.539
6. Guo Y, Shan Z, Ren H, Chen W. Dairy consumption and gastric cancer risk: a meta-analysis of epidemiological studies. *Nutr Cancer.* (2015) 67:555–68. doi: 10.1080/01635581.2015.1019634
7. Kong P, Cai Q, Geng Q, Wang J, Lan Y, Zhan Y, et al. Vitamin intake reduce the risk of gastric cancer: meta-analysis and systematic review of randomized and observational studies. *PLoS ONE.* (2014) 9:e116060. doi: 10.1371/journal.pone.0116060
8. Wu QJ, Yang Y, Wang J, Han LH, Xiang YB. Cruciferous vegetable consumption and gastric cancer risk: a meta-analysis of epidemiological studies. *Cancer Sci.* (2013) 104:1067–73. doi: 10.1111/cas.12195
9. Zhang Z, Xu G, Ma M, Yang J, Liu X. Dietary fiber intake reduces risk for gastric cancer: a meta-analysis. *Gastroenterology.* (2013) 145:113–20. doi: 10.1053/j.gastro.2013.04.001
10. Chen Y, Yu C, Li Y. Physical activity and risks of esophageal and gastric cancers: a meta-analysis. *PLoS ONE.* (2014) 9:e88082. doi: 10.1371/journal.pone.0088082
11. Li L, Gan Y, Wu C, Qu X, Sun G, Lu Z. Coffee consumption and the risk of gastric cancer: a meta-analysis of prospective cohort studies. *BMC Cancer.* (2015) 15:733. doi: 10.1186/s12885-015-1758-z
12. Han J, Jiang Y, Liu X, Meng Q, Xi Q, Zhuang Q, et al. Dietary fat intake and risk of gastric cancer: a meta-analysis of observational studies. *PLoS ONE.* (2015) 10:e0138580. doi: 10.1371/journal.pone.0138580
13. Song P, Lu M, Yin Q, Wu L, Zhang D, Fu B, et al. Red meat consumption and stomach cancer risk: a meta-analysis. *J Cancer Res Clin Oncol.* (2014) 140:979–92. doi: 10.1007/s00432-014-1637-z
14. Yang P, Zhou Y, Chen B, Wan HW, Jia GQ, Bai HL, et al. Overweight, obesity and gastric cancer risk: results from a meta-analysis of cohort studies. *Eur J Cancer.* (2009) 45:2867–73. doi: 10.1016/j.ejca.2009.04.019
15. La Torre G, Chiaradia G, Gianfagna F, De Lauretis A, Boccia S, Mannocci A, et al. Smoking status and gastric cancer risk: an updated meta-analysis of case-control studies published in the past ten years. *Tumori.* (2009) 95:13–22. doi: 10.1177/030089160909500103
16. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. *BMJ.* (2013) 346:f1326. doi: 10.1136/bmj.f1326
17. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure. *J Hum Hypertens.* (2002) 16:761–70. doi: 10.1038/sj.jhh.1001459
18. Dias-Neto M, Pintalhao M, Ferreira M, Lunet N. Salt intake and risk of gastric intestinal metaplasia: systematic review and meta-analysis. *Nutr Cancer.* (2010) 62:133–47. doi: 10.1080/01635580903305391
19. D'Elia L, Rossi G, Ippolito R, Cappuccio FP, Strazzullo P. Habitual salt intake and risk of gastric cancer: a meta-analysis of prospective studies. *Clin Nutr.* (2012) 31:489–98. doi: 10.1016/j.clnu.2012.01.003
20. Larsson SC, Orsini N, Wolk A. Processed meat consumption and stomach cancer risk: a meta-analysis. *J Natl Cancer Inst.* (2006) 98:1078–87. doi: 10.1093/jnci/djj301
21. Ge S, Feng X, Shen L, Wei Z, Zhu Q, Sun J. Association between habitual dietary salt intake and risk of gastric cancer: a systematic review of observational studies. *Gastroenterol Res Pract.* (2012) 2012:808120. doi: 10.1155/2012/808120
22. Shin JY, Kim J, Choi KS, Suh M, Park B, Jun JK. Relationship between salt preference and gastric cancer screening: an analysis of a nationwide survey in Korea. *Cancer Res Treat.* (2016) 48:1037–44. doi: 10.4143/crt.2015.333
23. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008
24. Wells G, Shea B, O'Connell D. *The Newcastle-Ottawa Scale (NOS) For Assessing the Quality of Nonrandomised Studies in Meta-Analyses*. Ottawa (ON): Ottawa Hospital Research Institute (2009). Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm
25. Ades AE, Lu G, Higgins JP. The interpretation of random-effects metaanalysis in decision models. *Med Decis Making.* (2005) 25:646–54. doi: 10.1177/0272989X05282643
26. Deeks JJ, Higgins JPT, Altman DG. Analyzing data and undertaking meta-analyses. In: Higgins J, Green S, editors. *Cochrane Handbook For Systematic Reviews of Interventions 5.0.1*. Oxford, UK: The Cochrane Collaboration (2008).
27. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
28. Tobias A. Assessing the influence of a single study in meta-analysis. *Stata Tech Bull.* (1999) 47:15–7.
29. Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. *Lancet.* (2011) 378:1297–305. doi: 10.1016/S0140-6736(11)60781-2
30. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629
31. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* (1994) 50:1088–101. doi: 10.2307/2533446

32. Nomura A, Grove JS, Stemmermann GN, Severson RK. A prospective study of stomach cancer and its relation to diet, cigarettes, and alcohol consumption. *Cancer Res.* (1990) 50:627–31.
33. Kneller RW, McLaughlin JK, Bjelke E, Schuman LM, Blot WJ, Wacholder S, et al. A cohort study of stomach cancer in a high-risk American population. *Cancer.* (1991) 68:672–8. doi: 10.1002/1097-0142(19910801)68:3<672::aid-cncr2820680339>3.0.co
34. Kato I, Tominaga S, Ito Y, Kobayashi S, Yoshii Y, Matsuura A, et al. A prospective study of atrophic gastritis and stomach cancer risk. *Jpn J Cancer Res.* (1992) 83:1137–42. doi: 10.1111/j.1349-7006.1992.tb02736.x
35. Kato I, Tominaga S, Matsumoto K. A prospective study of stomach cancer among a rural Japanese population: a 6-year survey. *Jpn J Cancer Res.* (1992) 83:568–75. doi: 10.1111/j.1349-7006.1992.tb00127.x
36. Galanis DJ, Kolonel LN, Lee J, Nomura A. Intakes of selected foods and beverages and the incidence of gastric cancer among the Japanese residents of Hawaii: a prospective study. *Int J Epidemiol.* (1998) 27:173–80. doi: 10.1093/ije/27.2.173
37. Knekt P, Järvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. *Int J Cancer.* (1999) 80:852–6. doi: 10.1002/(sici)1097-0215(19990315)80:6<852::aid-ijc9>3.0.co
38. McCullough ML, Robertson AS, Jacobs EJ, Chao A, Calle EE, Thun MJ, et al. Prospective study of diet and stomach cancer mortality in United States men and women. *Cancer Epidemiol Biomarkers Prev.* (2001) 10:1201–5.
39. Ngoan LT, Mizoue T, Fujino Y, Tokui N, Yoshimura T. Dietary factors and stomach cancer mortality. *Br J Cancer.* (2002) 87:37–42. doi: 10.1038/sj.bjc.6600415
40. van den Brandt PA, Botterweck AA, Goldbohm RA. Salt intake, cured meat consumption, refrigerator use and stomach cancer incidence: a prospective cohort study (Netherlands). *Cancer Causes Control.* (2003) 14:427–38. doi: 10.1023/A:1024979314124
41. Khan MM, Goto R, Kobayashi K, Suzumura S, Nagata Y, Sonoda T, et al. Dietary habits and cancer mortality among middle aged and older Japanese living in Hokkaido, Japan by cancer site and sex. *Asian Pac J Cancer Prev.* (2004) 5:58–65.
42. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA.* (2004) 291:187–94. doi: 10.1001/jama.291.2.187
43. Tokui N, Yoshimura T, Fujino Y, Mizoue T, Hoshiyama Y, Yatsuya H, et al. Dietary habits and stomach cancer risk in the JACC Study. *J Epidemiol.* (2005) 15:S98–108. doi: 10.2188/jea.15.S98
44. Sauvaget C, Lagarde F, Nagano J, Soda M, Koyama K, Kodama K. Lifestyle factors, radiation and gastric cancer in atomic-bomb survivors (Japan). *Cancer Causes and Control.* (2005) 16:773–80. doi: 10.1007/s10552-005-5385-x
45. Tran GD, Sun XD, Abnet CC, Fan JH, Dawsey SM, Dong ZW, et al. Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. *Int J Cancer.* (2005) 113:456–63. doi: 10.1002/ijc.20616
46. Kurosawa M, Kikuchi S, Xu J, Inaba Y. Highly salted food and mountain herbs elevate the risk for stomach cancer death in a rural area of Japan. *J Gastroenterol Hepatol.* (2006) 21:1681–6. doi: 10.1111/j.1440-1746.2006.04290.x
47. Shikata K, Kiyohara Y, Kubo M, Yonemoto K, Ninomiya T, Shirota T, et al. A prospective study of dietary salt intake and gastric cancer incidence in a defined Japanese population: the Hisayama study. *Int J Cancer.* (2006) 119:196–201. doi: 10.1002/ijc.21822
48. Larsson SC, Bergkvist L, Wolk A. Processed meat consumption, dietary nitrosamines and stomach cancer risk in a cohort of Swedish women. *Int J Cancer.* (2006) 119:915–9. doi: 10.1002/ijc.21925
49. González CA, Jakszyn P, Pera G, Agudo A, Bingham S, Palli D, et al. Meat intake and risk of stomach and esophageal adenocarcinoma within the European prospective investigation into cancer and nutrition (EPIC). *J Natl Cancer Inst.* (2006) 98:345–54. doi: 10.1093/jnci/djj071
50. Sjodahl K, Jia C, Vatten L, Nilsen T, Hveem K, Lagergren J. Salt and gastric adenocarcinoma: a population-based cohort study in Norway. *Cancer Epidemiol Biomarkers Prev.* (2008) 17:1997–2001. doi: 10.1158/1055-9965.EPI-08-0238
51. Kim J, Park S, Nam BH. Gastric cancer and salt preference: a population-based cohort study in Korea. *Am J Clin Nutr.* (2010) 91:1289–93. doi: 10.3945/ajcn.2009.28732
52. Takachi R, Inoue M, Shimazu T, Sasazuki S, Ishihara J, Sawada N, et al. Consumption of sodium and salted foods in relation to cancer and cardiovascular disease: the Japan public health center-based prospective study. *Am J Clin Nutr.* (2010) 91:456–64. doi: 10.3945/ajcn.2009.28587
53. Murata A, Fujino Y, Pham YM, Kubo T, Mizoue T, Tokui N, et al. Prospective cohort study evaluating the relationship between salted food intake and gastrointestinal tract cancer mortality in Japan. *Asia Pac J Clin Nutr.* (2010) 19:564–71.
54. Cross AJ, Freedman ND, Ren J, Ward MH, Hollenbeck AR, Schatzkin A, et al. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. *Am J Gastroenterol.* (2011) 106:432–42. doi: 10.1038/ajg.2010.415
55. Wang Z, Koh WP, Jin A, Wang R, Yuan JM. Composite protective lifestyle factors and risk of developing gastric adenocarcinoma: the Singapore Chinese health study. *Br J Cancer.* (2017) 116:679–87. doi: 10.1038/bjc.2017.7
56. Thapa S, Fischbach LA, Delongchamp R, Faramawi MF, Orloff M. Association between dietary salt intake and progression in the gastric precancerous process. *Cancers.* (2019) 11:467. doi: 10.3390/cancers11040467
57. Yoo JY, Cho HJ, Moon S, Choi J, Lee S, Ahn C, et al. Pickled vegetable and salted fish intake and the risk of gastric cancer: two prospective cohort studies and a meta-analysis. *Cancers.* (2020) 12:996. doi: 10.3390/cancers12040996
58. Duvall S, Tweedie R. A nonparametric "trim and fill" method for assessing publication bias in meta-analysis. *J Am Stat Assoc.* (2000) 95:89–98. doi: 10.1080/01621459.2000.10473905
59. Ganapathy E, Peramaiyan R, Rajasekaran D, Venkataraman M, Dhanapal S. Modulatory effect of naringenin on N-methyl-N'-nitro-N-nitrosoguanidine- and saturated sodium chloride-induced gastric carcinogenesis in male Wistar rats. *Clin Exp Pharmacol Physiol.* (2008) 35:1190–6. doi: 10.1111/j.1440-1681.2008.04987.x
60. Kato S, Tsukamoto T, Mizoshita T, Tanaka H, Kumagai T, Ota H, et al. High salt diets dose-dependently promote gastric chemical carcinogenesis in helicobacter pylori-infected *Mongolian gerbils* associated with a shift in mucin production from glandular to surface mucous cells. *Int J Cancer.* (2006) 119:1558–66. doi: 10.1002/ijc.21810
61. Fox JG, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances helicobacter pyloricolonization in C57BL/6 mice. *Cancer Res.* (1999) 59:4823–8.
62. Gaddy JA, Radin JN, Loh JT, Zhang F, Washington MK, Peek RM Jr, et al. High dietary salt intake exacerbates helicobacter pylori-induced gastric carcinogenesis. *Infect Immun.* (2013) 81:2258–67. doi: 10.1128/IAI.01271-12
63. Toyoda T, Tsukamoto T, Yamamoto M, Ban H, Saito N, Takasu S, et al. Gene expression analysis of a helicobacter pylori-infected and high-salt diet-treated mouse gastric tumor model: identification of CD177 as a novel prognostic factor in patients with gastric cancer. *BMC Gastroenterol.* (2013) 13:122. doi: 10.1186/1471-230X-13-122

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Association Between Fish Consumption and Muscle Mass and Function in Middle-Age and Older Adults

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Background: Sarcopenia, the age-related loss of skeletal muscle mass and function, represents a crucial risk factor for disability and mortality. Increasing intake of some nutrients, particularly protein and omega-3 fatty acids seems to be a promising strategy to augment muscle mass and function.

Objective: The purpose of this study was to assess the beneficial effects of fish consumption on muscle mass and function among middle-age and older adults.

Methods: Twenty-two adults aged 50–85 years participated in this study. Participants were asked to consume 150–170-g of fish for lunch twice a week for a 10-week period. During that period, participants were asked to maintain their normal diet and physical activity. Outcome measures included anthropometry, muscle mass, and muscle function. All these measures were assessed at baseline, week 5, and week 10. Repeated-measures analysis of variance was used to analyze statistical significance.

Results: Consuming fish twice a week for 10 weeks significantly increased the skeletal muscle mass and appendicular lean mass divided by height squared (ALM/h^2) ($p < 0.01$). Handgrip strength and gait speed < 0.8 m/s were also improved ($p < 0.01$) at week 10 compared with that at baseline.

Discussion: Consuming fish seems to improve muscle mass and function and may slow sarcopenia progression in middle-age and older adults.

Keywords: sarcopenia, fish, protein, omega-3 fatty acids, muscle mass, muscle function

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INTRODUCTION

The global population aged 60 years and above is growing faster than all younger age groups. In 2017, they numbered 962 million and is expected to increase to 2.1 and 3.1 billion by 2050 and 2100, respectively (1). Population aging is projected to have profound impacts on societies, underscoring the economic consequences that the healthcare sector is likely to face in many countries. A leading health issue in aging individuals is sarcopenia, which has significant clinical implications (2). The term "sarcopenia" was first defined as an age-related syndrome characterized by a progressive loss of skeletal muscle mass (SMM) (3). Recently, various expert groups from around the world have published consensus definitions and recommended using the presence of low muscle mass in combination with poor muscle function (muscle strength and physical performance) for diagnosing sarcopenia (4–6).

Interest in preventing and managing sarcopenia by improving muscle health is growing. Numerous studies have identified modifiable risk factors for sarcopenia, including diet (7, 8). Poor diet and nutrition status among older adults are often cited (9–11). Adopting a healthy diet can be an effective strategy to promote healthy aging. Seafood, including fish, is considered a part of a healthy diet, and consumption of at least 8 ounces of seafood per week is recommended (12). Furthermore, the recommended fish consumption is at least twice a week (13). Fish are a high-quality source of protein and omega-3 fatty acids, which are the dominant polyunsaturated fatty acids of fish oil. Fish are also rich in vitamins, including vitamin D. Protein and vitamin D are nutrients that have been consistently linked to sarcopenia determinants—muscle mass and muscle function (8). Indeed, protein is recognized as a key nutrient for better health among older adults (14). It provides amino acids that are required for muscle protein synthesis and acts as an anabolic stimulus directly effecting protein synthesis (8, 15). Vitamin D enhances muscle protein synthesis and boosts strength and balance (16, 17). However, the mechanisms by which vitamin D enhances muscle mass and function are not fully understood (8). In addition to protein and vitamin D, omega-3 fatty acids are suggested to be related to sarcopenia (18–20). Sarcopenia is proposed as an inflammatory state, and omega-3 fatty acids could be potent anti-inflammatory agents (18). In addition, fish contain vitamin E, which could be beneficial for maintaining muscle health. Considering the health benefits of the nutrients that fish contain, we hypothesized that nutritional intervention with fish would improve muscle mass and function. Therefore, this study was conducted to examine the beneficial effects of fish (i.e., red sea bass) consumption for 10 weeks on muscle mass and function among free-living middle-age and older adults.

METHODS

Study Participants

Twenty-two middle-age and older adults (eight men and 14 women), aged 50–85 years participated in this study. They were recruited via advertisements on social media and posters placed in several health centers in Riyadh, Saudi Arabia. Potential participants were excluded if they had any of the following conditions: disabilities, mental health issues, liver or kidney diseases, fish allergy, and body weight that was unstable in the last 3 months ($\geq \pm 3$ kg).

The study was conducted at two health centers in two regions, east and north of Riyadh, between December 2018 and May 2019. The participants voluntarily signed consent forms before inclusion in the study.

Calculation of Sample Size

A study (21) has demonstrated that muscle mass increases after an 8-week intervention with whey protein supplementation (from 59.6 ± 5.2 to 62.8 ± 5.2 kg). Therefore, 24 individuals were required to detect a difference in muscle mass with a power of 80% at a significance level of 0.05 using G*Power (Heinrich-Heine-Universität Düsseldorf, Brunsbüttel, Germany).

Study Design and Procedure

A within-subject experimental design was used in this study. Potential participants were screened using a general health questionnaire to assess their eligibility. They also completed the Mini-Nutritional Assessment Scale–Short Form (MNA-SF) to evaluate their nutritional status (22). Eligible participants were enrolled in a 10-week intervention. During the intervention period, participants were asked to consumed a filet portion of fish twice a week, with 3 days apart, and were asked to maintain their habitual diet and perform their normal daily physical activities. The investigator confirmed that all participants followed the instructions through weekly phone contact. Outcome measures including anthropometry (body weight; height; body mass index, BMI; waist circumference; hip circumference and waist-to-hip ratio), body composition (body fat, BF%; fat mass, FM; and SMM), and sarcopenia parameters (SMM index, SMI%; appendicular muscle mass divided by height squared, ALM/h^2; handgrip strength and gait speed) were assessed by a trained staff at baseline, week 5, and week 10 of the intervention.

Dietary Intake

The participants were instructed to complete a 3-day food record in which to record their food intake over 3 days (2 weekdays and 1 weekend day) to estimate their habitual dietary intake. Detailed instructions on how to complete the food diary were provided to each participant by a trained staff using semi-quantitative household measures. The diary was entered and analyzed using Food Processor (version 11.6, ESHA Research, Inc., Salem, OR). The habitual dietary intakes of the participants were compared with the 2015–2020 (8th edition) Dietary Guidelines (23). A filet portion of red sea bass was added to the participants habitual diet to calculate their intake with the additional fish intake. The total energy intake and macronutrients (carbohydrates, proteins, and fat) as grams and percentage contribution of energy were computed. Vitamins D and E were also assessed.

Intervention

The participants were instructed to consume one filet portion (150–170g) of red sea bass fish (also known as the barramundi sea bass) with their habitual diet at lunch twice a week for 10 weeks. The red sea bass was chosen in this study because it has been cultured in many Asian countries, including Saudi Arabia (24), has a good market price, and is preferred by consumers due to its delicate and mild-flavored white meat (25). In the current study, fish were supplied by the National Aquaculture Group (NAQUA, Riyadh, Saudi Arabia), transported to Zero Fat Restaurant (Riyadh, Saudi Arabia) in two batches—the first one was one day before the start of the study and the second one was after 5 weeks of the intervention—and stored at −20°C. One portion of filet was cooked and packaged for each participant and then delivered to participants' homes at lunch time as scheduled. The restaurant was advised to cook each portion of fish with a piece of lemon for approximately 20 min in an oven at 200°C. The amount of fish given to the participants was based on the Dietary Guidelines for Americans (Dietary Guidelines Advisory Committee 2015) (26). The investigator contacted the participants during the intervention period to follow up with their compliance to fish consumption.

Measurements

The following parameters were measured during the designated visits (baseline, week 5, and week 10) by the same investigator at the same time and under the same conditions according to the standard procedures. Body weight (kg) was measured to the nearest 0.1 kg using a digital scale. With the scale placed on a hard-flat surface and with the digital screen indicating zero, the participants were instructed to stand on the scale with both feet without shoes, wearing light clothes and with an empty bladder. Height was measured at inclusion, for the computation of BMI, barefoot to the nearest 0.1 cm using a stadiometer. BMI was calculated using the standard formula: body weight/height^2 (kg/m^2). The waist circumference was measured using a tape measure at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. The hip was measured at the widest portion of the buttocks. The waist-to-hip ratio was calculated as the ratio of the waist and hip circumferences.

Body composition was measured by bioelectric impedance analysis (BIA, Inbody 270, Cerritos, CA, USA). The participants stood on a balance scale in bare feet and held the conductive handles. After the 15-s measurement, the results were printed. The percentage of BF%, FM (kg), and SMM was obtained through body composition analysis.

SMI% was calculated by dividing the SMM by the body mass (SMM [kg]/body mass [kg] × 100) (27). ALM, which was defined as the sum of the lean masses of the arms and legs, was calculated using the following formula: ALM (kg)/ht^2 (m^2) (28).

Handgrip strength was assessed using the Jamar hand dynamometer (J00105, Lafayette Instrument Company, USA). The participants were instructed to stand with their arms and wrists stretched out at the sides of the body. Then, they were instructed to squeeze the handle as hard and as tightly as they could for 3–5 s. This measure was performed three times on each hand, with 60 s of recovery between each measurement. For the statistical analysis, the best value (in kg) among the repeated measurements was taken.

Physical performance was assessed using gait speed over a 4-m distance. The participants were instructed to walk at their usual speed with a static start without deceleration throughout a 4-meter straight line. Using a stopwatch, the time was recorded. Gait speed was expressed in meters per second (m/s) (29).

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (version 22; IBM Corp., Armonk, NY, USA). Data were checked for normality of distribution using the Kolmogorov–Smirnov test. Continuous data were presented as means ± standard deviations (SDs), and categorical data were presented as frequencies and proportions. The comparisons between the participants' habitual diet and DRI were performed using a paired *t*-test. One-way analysis of variance was performed to examine variations between the measured variables (anthropometry, body composition and sarcopenia parameters) across the study. Where significant main effects were determined, Fisher's least significant difference *post-hoc* test was conducted to determine pairwise differences between baseline, week 5, and week 10. *P*-values of <0.05 were used to denote statistical significance.

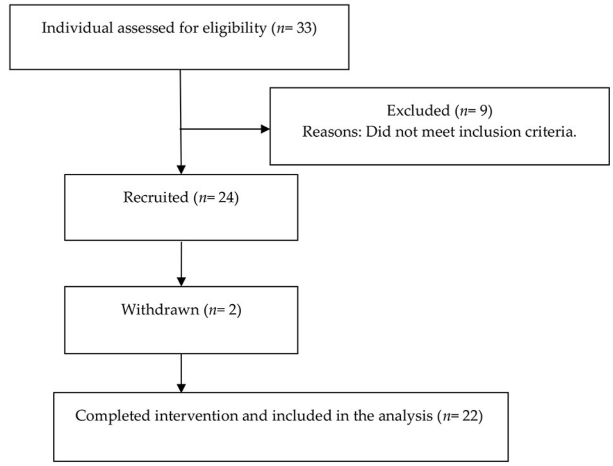

FIGURE 1 | Flow chart diagram of the study participants.

RESULTS

A total 33 middle-age and old adults (13 men and 20 women) were initially screened for eligibility, nine of whom (five men and four women) were excluded as they did not meet the inclusion criteria. Twenty-four participants (eight men and 16 women) enrolled in the study. Two women withdrew before the end of the study because they moved away. The final study sample consisted of 22 participants (eight men and 14 women). The flow chart of the study participants is displayed in **Figure 1**.

Baseline Characteristics

Table 1 shows the baseline general and clinical characteristics of the study participants. The mean age of the participants was 60.18 ± 9.0 years, based on the MNA-SF, the majority of the participants (91%) were non-malnourished.

Dietary Intake

The habitual dietary intakes for the study participants without and with the additional one portion of fish compared with DRI values among sex groups are presented in **Tables 2**, **3**.

The habitual dietary intakes for men showed a significantly lower intake of total energy, omega-3 fatty acids, vitamin D, and vitamin E than DRI values ($p < 0.05$). However, with the additional intake of one portion of fish, protein and fat intakes significantly increased above the DRI ($p < 0.05$), whereas energy intake and vitamin E remained significantly lower than DRI ($p < 0.05$). Omega-3 fatty acids and vitamin D increased and met the DRI value.

In women, a significantly lower intake of omega-3 fatty acids, vitamin D, and vitamin E in their habitual diet than DRI values ($p < 0.05$) were found. Alternatively, a significantly higher intake of protein than DRI values was noted ($p < 0.05$). The habitual dietary intake plus one portion of fish showed a significant increase in fat and omega-3 fatty acid compared with DRI values ($p < 0.05$). The protein and vitamin E intake remained significantly higher and lower, respectively, than DRI values ($p < 0.05$). Vitamin D increased and met the DRI values.

TABLE 1 | Baseline general and clinical characteristics of the study participants.

Characteristic	Total (n = 22)
Age (years)	60.18 ± 9.0
Height (cm)	160.61 ± 6.38
Body weight (kg)	64.25 ± 11.71
BMI (kg/m^2)	25.97 ± 4.82
Waist (cm)	90.50 ± 10.15
Hip (cm)	102.28 ± 8.60
Waist–hip ratio	0.89 ± 0.09
BF %	39.16 ± 9.22
FM (kg)	25.81± 8.96
SMM (kg)	20.62 ± 3.87
SMI (%)	31.21 ± 4.68
ALM/ht^2 (kg/m^2)	6.05 ± 1.02
Handgrip strength (kg)	20.63 ±7.11
Gait speed <0.8 m/s	1.35 ± 0.78
Nutritional status (MNA)	
Normal	20 (91)
Beginning of malnutrition	1 (4.5)
Malnutrition	1 (4.5)

Data are presented as mean ± standard deviation or frequency and percentage (%).ALM/ht², appendicular skeletal muscle divided by height squared; BMI, body mass index; BF%, body fat; FM, fat mass; SMI, skeletal muscle index; SMM, skeletal muscle mass; MNA, mini-nutritional assessment.

Outcome Measures

The effects of the additional intake of one portion per day of fish twice a week for 5 and 10 weeks on the study variables are presented in **Table 4**. Consuming a portion of fish twice a week significantly improved the SMM and ALM/ht^2 with a positive percentage of relative change at weeks 5 and 10 compared with those at baseline ($p < 0.05$). The waist circumference and waist–hip ratio significantly decreased at weeks 5 and 10 compared with those at baseline ($p < 0.05$). A significant decrease in BF% and FM was also noted at weeks 5 and 10 compared with those at baseline ($p < 0.05$). Handgrip strength at week 10 significantly increased compared with that at baseline ($p < 0.05$). In addition, a significant decrease in gait speed <0.8 m/s was observed at weeks 5 and 10 compared with that at baseline. Alternatively, all study variables did not significantly change among time points.

DISCUSSION

Our findings demonstrated that consuming fish twice a week for 5 and 10 weeks significantly enhanced the SMM, ALM/h^2, and gait speed < 0.8 m/s compared with those at baseline. In addition, handgrip strength significantly improved at week 10 compared with that at baseline. Consuming fish at least twice a week is recommended by the American Heart Association as part of a healthy diet (30). It has been reported that fish intake has, without doubt, a vital role in maintaining muscle mass (31). The mechanism behind the beneficial impacts of fish on muscle mass and function is likely multifactorial; including mechanisms of certain nutrients that fish has composed.

Protein provides essential amino acids that stimulate muscle protein synthesis (8). Adequate intake of protein can prevent skeletal muscle atrophy, impaired muscle growth, and functional

TABLE 2 | The habitual dietary intake of the study participants compared with DRI values among sex groups.

Parameters	Men (n = 8)			Women (n = 14)		
	habitual intake	DRI	P-value	habitual intake	DRI	P-value
Energy (Kcal)	1,570.2 ± 179.02	2,000	0.000	1,530.8 ± 319.74	1,600	0.244
Carbohydrates (g/d)	234.3 ± 50.57	130	0.001	208.9 ± 60.49	130	0.001
Carbohydrates (%kcal)	61.0	45–65	-	55.9	45–65	-
Protein (g/d)	60.6 ± 8.60	56	0.167	61.3 ± 13.30	46	0.001
Protein (%kcal)	15.8	10–35	-	16.4	10–35	-
Total fat (g/d)	43.4 ± 9.10	35.50	0.061	50.0 ± 22.10	35.5	0.065
Total fat (%kcal)	25.4	20–35	-	30.1	20–35	-
Saturated fat (g/d)	12.6 ± 4.00	16.7	0.062	15.8 ± 7.38	12	0.077
MUFA (g/d)	10.7 ± 6.40	17.7	0.063	12.1 ± 13.27	12.7	0.875
PUFA (g/d)	5.3 ± 3.20	13	0.063	5.8 ± 4.04	9.8	0.063
Omega-3 (g/d)	0.4 ± 0.20	1.6	0.001	0.5 ± 0.31	1.1	0.001
Vitamin D (μg/d)	1.2 ± 1.60	10	0.000	1.3 ± 2.61	10	0.000
Vitamin E (mg/d)	3.0 ± 1.80	15	0.000	2.6 ± 1.68	15	0.000

Data are presented as mean ± standard deviation. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

TABLE 3 | The habitual dietary intake with the additional portion of fish (∼160 g) for the study participants compared with DRI among sex groups.

	Men ($n = 8$)				Women ($n = 14$)	
	habitual intake	DRI	*P*-value	habitual intake	DRI	*P*-value
Energy (Kcal)	1,716.4 ± 179.02	2,000	0.002	1,680.0 ± 319.74	1,600	0.561
Carbohydrates (g/d)	234.3 ± 50.57	130	0.001	208.9 ± 60.49	130	0.001
Carbohydrates (%kcal)	55.4	45–65	-	50.6	45–65	-
Protein (g/d)	90.1 ± 8.60	56	0.000	90.9 ± 13.30	46	0.000
Protein (%kcal)	21.3	10–35	-	22.0	10–35	-
Total fat (g/d)	46.6 ± 9.10	35.5	0.010	53.2 ± 22.10	35.5	0.010
Total fat (%kcal)	24.8	20–35	-	29.0	20–35	-
Saturated fat (g/d)	13.4 ± 4.00	16.7	0.355	16.6 ± 7.40	12	0.136
MUFA (g/d)	11.3 ± 6.40	17.7	0.067	12.8 ± 13.30	12.7	0.978
PUFA (g/d)	5.3 ± 3.20	13	0.081	7.0 ± 4.00	9.8	0.023
Omega3 (g/d)	1.6 ± 0.20	1.6	0.917	1.7 ± 0.30	1.1	0.000
Vitamin D (μg/d)	10.1 ± 1.60	10	0.839	10.3 ± 2.60	10	0.684
Vitamin E (mg/d)	4.3 ± 1.80	15	0.000	4.0 ± 1.70	15	0.000

Data are presented as mean ± standard deviation. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

TABLE 4 | Relative changes at weeks 5 and 10 associated with the additional intake of one portion (150–170 g) of fish per day in the study participants.

$N = 22$	Baseline	Week 5	Week 10	Relative change from baseline (%)	
				Week 5	Week 10
Body weight (kg)	64.25 ± 11.71	64.17 ± 11.72	63.89 ± 11.47	−0.12	−0.56
BMI (kg/m^2)	25.97 ±4.82	25.99 ± 4.93	25.88 ± 4.87	0.07	−0.34
Waist (cm)	90.50 ± 10.15	87.83 ± 9.83	86.72 ± 6.89	−2.99*	−4.17**
Hip (cm)	102.28 ± 8.60	100.56 ± 7.53	100.44 ± 7.85	− 1.68	− 1.79
Waist–hip ratio	0.89 ± 0.09	0.88 ± 0.10	0.86 ± 0.08	−1.12*	−3.37*
BF %	39.16 ± 9.22	37.22 ± 11.26	37.14 ± 11.04	−4.95*	−5.15*
FM (kg)	25.81 ± 8.96	24.80 ± 9.67	24.64 ± 9.53	−3.91*	−4.53*
SMM (kg)	20.62 ± 3.87	21.14 ± 3.81	21.07 ± 3.67	2.52*	2.18*
SMI (%)	31.21 ± 4.68	31.84 ± 5.99	31.82 ± 5.75	2.01	2.00
ALM/ht^2 (kg/m^2)	6.05 ± 1.02	6.19 ± 1.01	6.21 ± 0.98	2.31*	2.64*
Handgrip strength (kg)	20.63 ± 7.11	20.82 ± 8.65	21.86 ± 9.11	1.00	5.96*
Gait speed <0.8 m/s	1.35 ± 0.78	1.13 ± 0.67	0.97 ± 0.43	−16.29*	−28.14*

*Data are presented as mean ± standard deviation. *$P < 0.05$ significantly different from the baseline; **$P < 0.01$ significantly different from the baseline. ALM/ht², appendicular skeletal muscle divided by height squared; BMI, body mass index; BF%, body fat; FM, fat mass; SMI, skeletal muscle index; SMM, skeletal muscle mass.*

decline (32). A growing body of evidence suggests that older adults need a higher protein intake and shows that higher protein intake is favorable in maintaining their health and functionality (33–35). The main concern for older age is that the anabolic response to protein intake might be blunted; thus, increasing their intake of protein can help sustain nitrogen balance and prevent loss of muscle mass and function (14, 35). In this study, the addition of fish to the participants' normal diet led to an increase in protein intake above the DRI values for both men and women, which might help enhance protein synthesis, improving muscle mass and function. Higher intake of animal-protein foods (i.e., meat, fish, and eggs) was associated with the preservation of muscle mass and function among older adults (36). Although no interventional study has been conducted on humans to assess the effects of fish protein alone on muscles, a study on rats has shown that fish protein intake increases skeletal muscle weight (37). A previous cross-sectional study has reported an increase in handgrip strength of 0.43 kg in men and 0.48 kg in women for each additional portion of fatty fish consumed per week (38).

Vitamin D deficiency is highly prevalent in Saudi Arabia across all demographics and considered as a public health concern in the country (39). Consuming foods rich in vitamin D, such as fish, should be encouraged (20). In the current study, intake of vitamin D was below the DRI values; however, with the additional portion of fish, vitamin D levels increased and met the DRI values. Vitamin D has been identified as necessary for normal development and growth of muscle fibers, with its deficiency adversely influencing muscle function (40). Food consumption combined protein and other essential nutrients for the maintenance of muscle mass including vitamin D (i.e., fish) may improve muscle mass and function more effectively. A study involving older adults with sarcopenia has shown that the combined supplementation of whey protein and vitamins D and E significantly enhances the relative SMI and muscle strength (41). Bauer et al. have reported that a vitamin D oral supplementation with leucine-enriched whey protein improves muscle mass and lower-extremity function in older adults (42).

Fish is also an important source of omega-3 fatty acids (43), and particular attention has been focused on the role of omega-3 fatty acids on muscle mass and function. Our omega-3 fatty acids findings conform to previous studies wherein dietary supplementation with fish omega-3 fatty acids improves muscle protein degradation and increases muscle strength in older adults (38, 44). It has been reported that adding 4 g/day of fish omega-3 fatty acids for 8 weeks to the normal diet of older adults increased the acute amino acid-induced activation of the mTOR-p70s6k signaling pathway and muscle protein synthesis (45). Omega-3 fatty acids can diminish muscle decline by increasing the functional capacity by growing the intracellular metabolic signal in older adults (46, 47). Evidence shows that inflammation could play a role in the genesis of sarcopenia (48) and the anti-inflammatory actions of omega-3 fatty acids may also play a role in preventing sarcopenia (38). Intake of sufficient amount of omega-3 fatty acids could represent an effective nutritional therapy for individuals with sarcopenia (20).

Although vitamin E remained below the DRI values even after adding the fish portion, it might be that the relatively small increase in its level in combination with the increase in omega-3 intake during the intervention improved muscle mass and function more effectively. The association between vitamin E and inflammation has gained popularity recently. Vitamin E has antioxidative capacity, and supplementation of vitamin E was shown to protect against oxidative stress and inflammation (49). A study by Meydani et al. (50) has found that vitamin E supplementation lowers the expression of oxidative stress markers following a downhill run among adults.

No significant differences were observed between the outcome measurements between weeks 5 and 10, however, frequent fish consumption seems to improve sarcopenia parameters. It should be mentioned that the available data in the literature are inadequate to determine whether all types of fish, as a food, have the same beneficial impacts on muscles. More observational and intervention studies are warranted in this area. In this study, no significant differences in body weight were observed. One portion of fish provided an additional 145–165 kcal, a relatively minor contribution to the daily intake. Significant decreases in FM, BF%, waist circumference, and waist–hip ratio were observed at weeks 5 and 10 compared with those at baseline. Arciero et al. (51) have reported that consuming high-protein meals more frequently (6×/day) decreased abdominal fat and increased muscle mass.

In the current study, although all participants reported that their body weight was stable in the last three months, the self-reported energy intakes for men were significantly lower than DRI values. Under-reporting of daily energy intake is a common and acknowledged source of measurement error in the assessment of food intake (52, 53). It has been reported that older adults under-report more than younger populations (54) and men in our study were older than the women.

It should be mentioned that the sample size was not calculated to detect differences by sex. The effects of sex on the outcome measures should be considered in future nutritional intervention studies. One of the strengths of this study is the area of research itself. Further, to the best of our knowledge, this is the first intervention study that evaluated the beneficial impact of fish as a food on sarcopenia parameters. Nonetheless, this study has some limitations that should be considered when interpreting its results. The primary limitation was the lack of a control group due to the lack of responses to the study announcement and this limits the strength of our findings. Also, the study participants were free-living, and we did not control their dietary intakes and physical activity, even though they were instructed to maintain their habitual diet and physical activity during the intervention period. In addition, middle-age and older adult participants were included in this study which may affect the homogeneity in the participants' age. The relatively small sample size was also acknowledged. Finally, this study only involved independently living participants thus, the beneficial impact of fish consumption on older adults who have an instant need for keeping mobility and physical function are warranted.

In conclusion, this study shows that consuming fish (i.e., red sea bass) twice a week for 10 weeks could improve sarcopenia parameters and muscle mass and function in middle-age and older men and women. These findings suggest that fish have an important role on muscle health by different mechanisms, including effects on muscle protein synthesis, inflammation, and oxidative stress. Intakes of protein, vitamin D, omega-3 fatty acids, and antioxidants could all be crucial for optimal muscle function and may slow the progression of sarcopenia. Further well-designed interventional studies with fish intake among older adults are recommended to confirm the beneficial impact of fish on muscle health.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Institutional Review Board at King Saud University (Reference No. 19-0834/ IRB). All procedures were conducted according to the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MHA and MMA: conceptualization and data interpretation. MMA: investigation and data analysis. MHA: visualization, supervision, writing the original draft of the manuscript, and review and editing. Both authors have read and approved the final manuscript.

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REFERENCES

1. United Nations, Department of Economic and Social Affairs, Population Division. *World Population Prospects: The 2017 Revision, Key Findings and Advance Tables.* Working Paper No. ESA/P/WP/248. New York, NY (2017).
2. Senior HE, Henwood TR, Beller EM, Mitchell GK, Keogh JPrevalence W, and risk factors of sarcopenia among adults living in nursing homes. *Maturitas.* (2015) 82:418–23. doi: 10.1016/j.maturitas.2015.08.006
3. Rosenberg IH. Sarcopenia: origins and clinical relevance. *J Nutr.* (1997) 127:990S—1S. doi: 10.1093/jn/127.5.990S
4. Cruz-Jentoft. European Working group on sarcopenia in older people: Sarcopenia: European consensus on definition and diagnosis. Report of the European workign group on sarcopenia in older people age. *Ageing.* (2010) 39:412–23. doi: 10.1093/ageing/afq034
5. Chen L-K, Liu L-K, Woo J, Assantachai P, Auyeung T-W, Bahyah KS, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc.* (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025
6. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences International working group on sarcopenia. *J Am Med Dir Assoc.* (2011) 12:249–56. doi: 10.1016/j.jamda.2011.01.003
7. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr.* (2008) 87:150–5. doi: 10.1093/ajcn/87.1.150
8. Robinson SM, Reginster J-Y, Rizzoli R, Shaw S, Kanis JA, Bautmans I, et al. Does nutrition play a role in the prevention and management of sarcopenia? *Clin Nutr.* (2018) 37:1121–32. doi: 10.1016/j.clnu.2017.08.016
9. Kaiser MJ, Bauer JM, Rämsch C, Uter W, Guigoz Y, Cederholm T, et al. Frequency of Malnutrition in Older Adults: A Multinational Perspective Using the Mini Nutritional Assessment. *J Am Geriatr Soc.* (2010) 58:1734–8. doi: 10.1111/j.1532-5415.2010.03016.x
10. Rasheed S, Woods RMalnutrition T, and quality of life in older people: A systematic review and meta-analysis. *Ageing Res Rev.* (2013) 12:561–6. doi: 10.1016/j.arr.2012.11.003
11. Hengeveld LM, Wijnhoven HA, Olthof MR, Brouwer IA, Harris TB, Kritchevsky SB, et al. Prospective associations of poor diet quality with long-term incidence of protein-energy malnutrition in community-dwelling older adults: the Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr.* (2018) 107:155–64. doi: 10.1093/ajcn/nqx020
12. U.S Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 27. (2014). Available online at: http://www.ars.usda.gov/nutrientdata
13. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation.* (2006) 114:82-96. doi: 10.1161/CIRCULATIONAHA.106.176158
14. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. *Clin Nutr.* (2008) 27:675–84. doi: 10.1016/j.clnu.2008.06.008
15. Kim J-S, Wilson JM, Lee S-R. Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants. *J Nutr Biochem.* (2010) 21:1–13. doi: 10.1016/j.jnutbio.2009.06.014
16. Muir SW, Montero-Odasso M. Effect of Vitamin D Supplementation on Muscle Strength, Gait and Balance in Older Adults: A Systematic Review and Meta-Analysis. *J Am Geriatr Soc.* (2011) 59:2291–300. doi: 10.1111/j.1532-5415.2011.03733.x
17. Latham NK, Anderson CS, Reid IR. Effects of Vitamin D Supplementation on Strength, Physical Performance, and Falls in Older Persons: A Systematic Review. *J Am Geriatr Soc.* (2003) 51:1219–26. doi: 10.1046/j.1532-5415.2003.51405.x
18. Robinson S, Cooper C, Aihie Sayer A. Nutrition and sarcopenia: A review of the evidence and implications for preventive strategies. *J Aging Res.* (2012) 2012:510801. doi: 10.1155/2012/510801
19. Mithal A, Bonjour J-P, Boonen S, Burckhardt P, Degens H, Fuleihan GEH, et al. Impact of nutrition on muscle mass, strength, and performance in older adults. *Osteoporosis Int.* (2013) 24:1555–66. doi: 10.1007/s00198-012-2236-y
20. Calvani R, Miccheli A, Landi F, Bossola M, Cesari M, Leeuwenburgh C, et al. Current nutritional recommendations and novel dietary strategies to manage sarcopenia. *J Frailty Aging.* (2013) 2:38–53. doi: 10.14283/jfa.2013.7
21. Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO, Wilson SMC, et al. The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J.* (2013) 12:86. doi: 10.1186/1475-2891-12-86
22. Guigoz Y, Vellas B, Garry PJ. Assessing the nutritional status of the elderly: The Mini Nutritional Assessment as part of the geriatric evaluation. *Nutr Rev.* (1996) 54:S59. doi: 10.1111/j.1753-4887.1996.tb03793.x
23. U.S. Department of Health and Human Services and U.S. Department of Agriculture. *2015 – 2020 Dietary Guidelines for Americans.* 8th ed. (2015). Available online at: https://health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015
24. Jerry DR. Biology and culture of Asian seabass Lates calcarifer. CRC Press. (2013). doi: 10.1201/b15974
25. Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Dietary administration of astaxanthin improves feed utilization, growth performance and survival of Asian seabass, Lates calcarifer (Bloch, 1790). *Aquac Nutr.* (2019) 25:1410–21. doi: 10.1111/anu.12961
26. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, cardiovascular disease. *Circulation.* (2002) 106:2747–57. doi: 10.1161/01.CIR.0000038493.65177.94
27. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc.* (2002) 50:889–96. doi: 10.1046/j.1532-5415.2002.50216.x
28. Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J, et al. Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. *Am J Clin Nutr.* (1990) 52:214–8. doi: 10.1093/ajcn/52.2.214
29. Pasma JH, Stijntjes M, Ou SS, Blauw GJ, Meskers CGM, Maier AB. Walking speed in elderly outpatients depends on the assessment method. *Age.* (2014) 36:9736. doi: 10.1007/s11357-014-9736-y
30. American Heart Association. Available online at: https://www.heart.org/

31. Rondanelli M, Rigon C, Perna S, Gasparri C, Iannello G, Akber R, et al. Novel insights on intake of fish and prevention of sarcopenia: all reasons for an adequate consumption. *Nutrients.* (2020) 12:307. doi: 10.3390/nu12020307
32. Deer RR, Volpi E. Protein intake and muscle function in older adults. *Curr Opin Clin Nutr Metab Care.* (2015) 18:248–53. doi: 10.1097/MCO.0000000000000162
33. Gaffney-Stomberg E, Insogna KL, Rodriguez NR, Kerstetter JE. Increasing Dietary Protein Requirements in Elderly People for Optimal Muscle and Bone Health. *J Am Geriatr Soc.* (2009) 57:1073–9. doi: 10.1111/j.1532-5415.2009.02285.x
34. Morley JE, Argiles JM, Evans WJ, Bhasin S, Cella D, Deutz NEP, et al. Nutritional recommendations for the management of sarcopenia. *J Am Med Dir Assoc.* (2010) 11:391–6. doi: 10.1016/j.jamda.2010.04.014
35. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc.* (2013) 14:542–59. doi: 10.1016/j.jamda.2013.05.021
36. Bradlee ML, Mustafa J, Singer MR, Moore LL. High-protein foods and physical activity protect against age-related muscle loss and functional decline. *J Gerontol A Biol Sci Med Sci.* (2017) 73:88–94. doi: 10.1093/gerona/glx070
37. Kawabata F, Mizushige T, Uozumi K, Hayamizu K, Han L, Tsuji T, et al. Fish protein intake induces fast-muscle hypertrophy and reduces liver lipids and serum glucose levels in rats. *Biosci Biotechnol Biochem.* (2015) 79:109–16. doi: 10.1080/09168451.2014.951025
38. Robinson SM, Jameson KA, Batelaan SF, Martin HJ, Syddall HE, Dennison EM, et al. Diet and its relationship with grip strength in community-dwelling older men and women: the hertfordshire cohort study. *J Am Geriatr Soc.* (2008) 56:84–90. doi: 10.1111/j.1532-5415.2007.01478.x
39. N.M. Al-Daghri. Vitamin D in Saudi Arabia: Prevalence,distribution and disease associations. *J Steroid Biochem Mol Biol.* (2018) 175:102–7. doi: 10.1016/j.jsbmb.2016.12.017
40. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: National Academies Press (US) (2011).
41. Bo Y, Liu C, Ji Z, Yang R, An Q, Zhang X, et al. A high whey protein, vitamin D and E supplement preserves muscle mass, strength, and quality of life in sarcopenic older adults: A double-blind randomized controlled trial. *Clin Nutr.* (2019) 38:159–64. doi: 10.1016/j.clnu.2017.12.020
42. Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc.* (2015) 16:740–7. doi: 10.1016/j.jamda.2015.05.021
43. Nichols PD, Glencross B, Petrie JR, Singh SP. Readily available sources of long-chain omega-3 oils: is farmed Australian seafood a better source of the good oil than wild-caught seafood? *Nutrients.* (2014) 6:1063–79. doi: 10.3390/nu6031063
44. Gordon Smith I, Atherton P, Dominic NR, Mohammed BS, Rankin D, Michael J et al. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia–hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci.* (2011) 121:267–78. doi: 10.1042/CS20100597
45. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am J Clini Nutr.* (2011) 93:402–12. doi: 10.3945/ajcn.110.005611
46. Smith GI, Julliand S, Reeds DN, Sinacore DR, Klein S, Mittendorfer B. Fish oil–derived n−3 PUFA therapy increases muscle mass and function in healthy older adults1. *Am J Clin Nutr.* (2015) 102:115–22. doi: 10.3945/ajcn.114.105833
47. Tachtsis B, Camera D, Lacham-Kaplan O. Potential roles of n-3 PUFAs during skeletal muscle growth and regeneration. *Nutrients.* (2018) 10:309. doi: 10.3390/nu10030309
48. Little JP, Phillips SM. Resistance exercise and nutrition to counteract muscle wasting. *Appl Physiol Nutr Metab.* (2009) 34:817–28. doi: 10.1139/H09-093
49. Khor SC, Abdul Karim N, Wan Ngah WZ, Y.A. Mohd Yusof, Makpol S. Vitamin E in sarcopenia: current evidences on its role in prevention and treatment. *Oxid Med Cell Longev.* (2014) 2014: 914853. doi: 10.1155/2014/914853
50. Meydani M, Evans W, Handelman G, Biddle L, Fielding R, Meydani S, et al. Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. *Am J Physiol Regul Integr Comp Physiol.* (1993) 264:R992–8. doi: 10.1152/ajpregu.1993.264.5.R992
51. Arciero PJ, Ormsbee MJ, Gentile CL, Nindl BC, Brestoff JR, Ruby M. Increased protein intake and meal frequency reduces abdominal fat during energy balance and energy deficit. *Obesity.* (2013) 21:1357–66. doi: 10.1002/oby.20296
52. Livingstone MBE, Black AE. Markers of the validity of reported energy intake. The Journal of nutrition (2003) 133:895S—920S. doi: 10.1093/jn/133.3.895S
53. Poslusna K, Ruprich J, de Vries JH, Jakubikova M, van't Veer P. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. *Br J Nutr.* (2009) 101:S73–S85. doi: 10.1017/S0007114509990602
54. Pfrimer K, Vilela M, Resende CM, Scagliusi FB, Marchini JS, Lima NKC, et al. Under-reporting of food intake and body fatness in independent older people: a doubly labelled water study. *Age and Ageing.* (2014) 44:103–8. doi: 10.1093/ageing/afu142

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Shifting to a Sustainable Dietary Pattern in Iranian Population: Current Evidence and Future Directions

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The need for a shift in diet toward a more sustainable one has reached an urgency in certain regions, including Iran, due to more rapid climate change and a higher level of vulnerability. This study was undertaken to identify and summarize available data on changes required in the current Iranian diet to make it more sustainable and the extent to which current policies in the country have addressed such a shift. In this study, PubMed, Scopus, and Web of science, as well as Iranian scientific search engines, including Scientific Information Database and Magiran, were systematically searched from January 1990 to July 2021. A total of 11 studies and policy analyses were included in this study. Based on the findings, moving Iranian diet toward sustainability will require increase in consumption of dairy, fruits, vegetables, cereals, poultry, and legumes and decrease in consumption of bread, rice, pasta, red meat, eggs, fats, sugars, and sweets. There has been a great deal of effort and investment on policies and strategies to decrease the amount of sugar, salt, and fat (specifically trans-fatty acids) in the Iranian diet, which makes it more sustainable healthwise. Several policies and programs have been implemented to tackle non-communicable diseases (NCDs) by reducing access to unhealthy foods, which is in line with health dimension of a sustainable diet. However, there is almost no direct address to ecological aspect of sustainable diet in the food and nutrition policy documents in the ccountry. Development of an enabling environment to a sustainable diet will require policy and actions to improve public awareness, support study to provide evidence and identify possible alternatives, and plan and implement interventions/programs to promote and facilitate healthy and sustainable diets.

Keywords: sustainable diet, dietary change, environmental footprint, nutrition, Iran

INTRODUCTION

Over the last decades, nations have experienced urbanization and consequently transformational adaptation of food systems that have resulted in substantial change in their traditional diet known as nutrition transition (1). These dietary changes have been accompanied by a higher intake of calorie and resource-intensive foods, e.g., animal products (meats and dairy), vegetable oil, and sugar (2). Based on the changes that have been happening, it is foreseen that there is 70% gap, called "food gap," between the crop calories available in 2006 and expected calorie demand in 2050 (3). One of the options for closing this gap by 2050, as stated in the sustainable development goals (SDGs), is through shifts in food demand including shifting diets, reducing food waste, and avoiding competition from bioenergy (4). Such a shift has the potential to impact health of people and environment.

There is a growing literature on health co-benefits of sustainable diets at global, regional, national, and subnational (modeling) studies estimating the potential impact of dietary change on both the environment and health (4). According to the Food and Agriculture Organization (FAO) definition, "sustainable diets are those diets with low environmental impacts, which contribute to food and nutrition security and healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable, nutritionally adequate, safe, and healthy, while optimizing natural and human resources" (5). Recent studies have shown that diets comprising reduced animal sources (specifically reduced red and processed meat) and high levels of plant-based foods including fruits and vegetables are not only associated with decrease in non-communicable diseases (NCDs) (6, 7), but can result in lower environmental footprints (8, 9).

The need for a shift toward a more sustainable diet may have more urgency in certain regions. An example is Iran, a middle-income country located in the Middle East that is mostly an arid and semi-arid region where climate change is occurring more rapidly. It is expected that the amount of precipitation in this region drop by 20% within the next century, which makes the region more vulnerable (10). In addition, over the last four decades, due to industrialization and rapid urbanization, nutrition transition and major changes in the food system in the country have occurred that put further pressure on the environment as well as health of the population (11). Therefore, considering the urgency of the problem and the fact that there is not one sustainable diet, there is a need to define the most sustainable choices based on food availability and dietary pattern in each region (12).

There are relatively limited data available on sustainability aspects of the Iranian diet. This study was undertaken to identify and summarize available data on changes required in the current Iranian diet to make it more sustainable and the extent to which current policies in the country has addressed such a shift.

METHODS

Conceptual Framework

Sustainable diets are diets that consider how the food system influences health, environmental outcomes, and vice versa (5). Therefore, sustainable healthy diets are dietary patterns that promote all the dimensions of health and well-being of an individual; in the meantime, sustainable healthy diets have low environmental pressure and impact and are accessible, affordable, safe, equitable, and culturally acceptable (5). Sustainable healthy diets aim to achieve optimal growth and development of all the individuals and support functioning and physical, mental, and social well-being at all the life stages for present and future generations; contribute to preventing all the forms of malnutrition (i.e., undernutrition, micronutrient deficiency, overweight, and obesity); reduce the risk of diet-related NCDs and support the preservation of biodiversity and planetary health (12). In other words, sustainable healthy diets must combine different dimensions of sustainability to avoid unintended consequences, thus shifting from "current" to more "sustainable diets" that could serve as both a climate mitigation strategy and improved population health. For these reasons, shifting to a sustainable diet has been proposed as one of the core strategies to achieve SDGs (5).

Despite these facts, reaching a sustainable diet is a complex process that requires taking several steps to consider nutrient recommendations as well as environmental, social/cultural, and economic sustainability. **Figure 1** presents a conceptual framework adapted from "Guiding Principles for Sustainable Healthy Diets" developed by the FAO and the World Health Organization (WHO) (12) as a basis for this study. Based on this framework, in order to shift to a sustainable diet, various actions with respect to health, environmental, and sociocultural aspects of diet, including changing intake of different food groups, should be taken.

METHODOLOGY

Identifying the Study Question

This study aimed to answer the following questions:

- To what extent the topic of "sustainable diets" has been addressed in studies conducted in Iran?
- What is the most widely used definition of a sustainable diet in Iranian studies and which dimension of diet sustainability has been studied most?
- To what extent are diets in Iran in accordance with the principles of a sustainable diet?
- Has the issue of sustainable diet been considered/addressed in food and nutrition policies/guidelines in Iran?
- What policies and programs are needed in moving toward a sustainable diet in Iran?

Identifying Relevant Studies

We systematically searched articles related to sustainability of diet/nutrition/food system in Iran, using PubMed, Scopus, Web of science, and two Persian scientific search engines: Scientific Information Database (SID) (www.sid.ir, accessed on 15 July 2021) and Magiran (www.magiran.com, accessed on 15 July 2021).

The literature search was adapted to the databases and included the following subject heading, terms, and keywords: (carbon footprint or water footprint or land footprint or environment or sustainability or resilient or biodiverse or ecologic or life cycle analysis or global warming or climate or greenhouse gas (GHG) or greenhouse) and (food or diet or nutrition or consumption) and (Iran). We limited the search to the following dates: 1st January 1990 to 15th July 2021

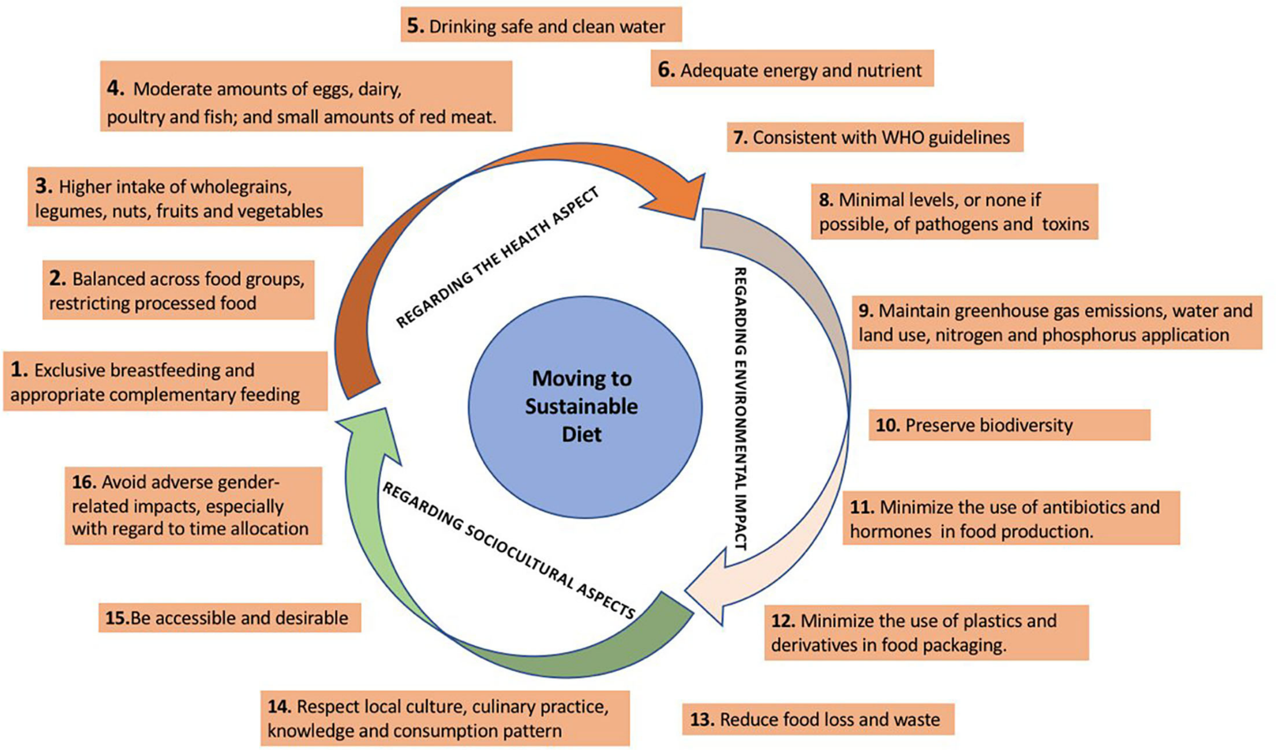

FIGURE 1 | Conceptual framework of shift to a sustainable diet (12).

and applied no language restriction. Additional references were identified by searching the gray literature and hand searching the reference lists of the included articles.

Study Selection

All the articles (in Farsi or English) that had information related to sustainable diet in Iran were included in this study. Studies that were related to sustainability issues other than food, nutrition, and/or diet or conducted in countries other than Iran were excluded. Screening of titles and abstracts was followed by full-text screening and data extraction.

Charting the Data

The data extraction table was designed to record the following variables: year of publication, aims, food item(s)/diet, sustainability dimensions measured, changes suggested in the diet, and finding(s).

Collating, Summarizing, and Reporting Results

Included studies were reviewed and findings with respect to the questions of the study were collected and summarized. In all the stages, two of the authors (SRS and NO) held regular meetings to discuss findings and reach a consensus about the management of the findings.

RESULTS

As illustrated in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram (**Figure 2**), 226 records were identified, of which 127 records were screened and excluded due to duplication. By reviewing the title and abstract, 42 records satisfied eligible criteria that were chosen for their full text to be read. Finally, 11 studies met the inclusion criteria and were included in this study (**Table 1**).

"Sustainable Diets" Definition and Measurement in Studies in Iran

The results indicated that "sustainable diet" is a topic that is being addressed by researchers in Iran in the recent years. Except for one study by Rahmani et al. in 2011, all the included studies had been conducted between 2017 and 2021 (13). Almost all the included studies were focused on the effect of dietary consumption change on the sustainability of diet (13, 14, 16–23), except one (15) that assessed the compliance of national nutrition policy with the sustainable diet framework. Of 10 studies on measuring sustainability dimensions of diet and changing it toward sustainability, 7 studies had focused on total dietary consumption (13, 17–20, 22, 23), one on a campus lunch menu (21), one on 14 important crops in Iran (16), and one compared the sustainability of traditional Iranian dishes with western dishes (14).

With respect to different dimensions of the sustainable diet, all the 10 studies had considered one or more of the environmental

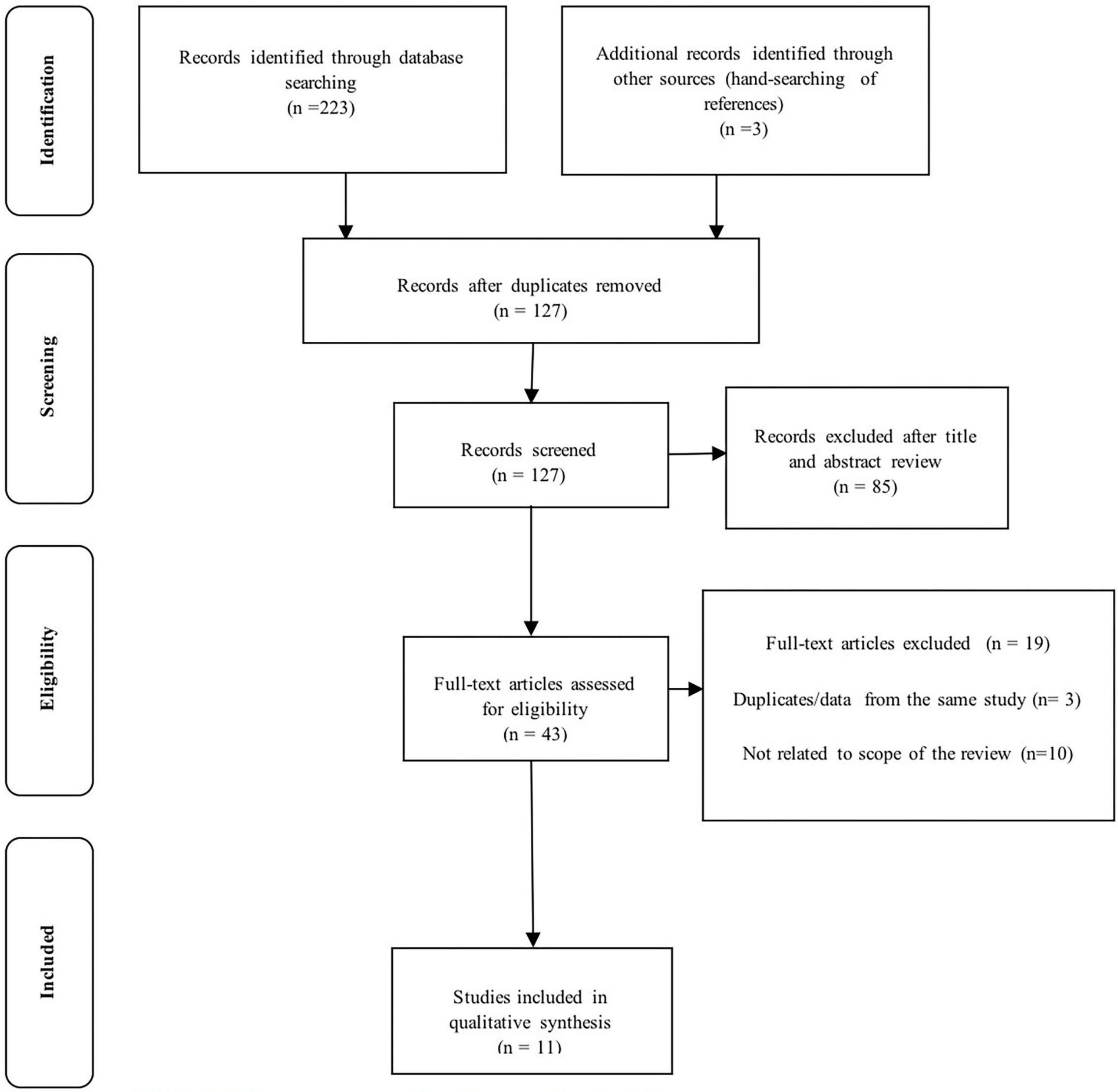

FIGURE 2 | The PRISMA flow diagram for the study selection process.

dimensions, specifically water footprint (14, 16–20, 23) and carbon footprint (13, 14, 17, 20–23). However, energy (17) and land footprint (21, 23) each were measured only in one and two studies, respectively. In addition, seven studies evaluated fulfillment of nutritional recommendations (17–23) and cost and cultural acceptance of diet were considered in five (13, 17, 20, 21, 23) and six (18–23) studies, respectively.

Dietary Changes Required to Move Toward a Sustainable Diet in Iran

The main approach used to identify what changes are needed to make the diet more sustainable was through comparing available data on Iranian food consumption and/or acquisition including food balance sheet data (13), household food expenditure (19, 20, 23), and food intake data (17, 18, 21, 22) with a designed and/or recommended sustainable diet. In order to design a sustainable diet, an optimizing method, i.e., linear programming (18, 19, 22), goal programming (20, 21, 23), or a recommended reference diet (13, 17) was used. The included studies based on their main purpose can be classified into three groups proposing a sustainable diet: (1) to reduce carbon footprint (13, 22); (2) to reduce water footprint (16, 18, 19); and (3) to fulfill nutritional, environmental, and economical

TABLE 1 | Summary of characteristics of studies on sustainable diet in Iran (1990–2021).

First author (year)	Aim	Food item(s)/diet	Sustainable diet dimension(s) measured	Suggested change(s) in diet	Finding(s)
Rahmani et al. (13)	To determine the impacts of dietary changes on the Iranian economy and on the environmental load.	Total diet, based on food balance sheet	• Carbon FP • Cost • Nutritional value	– Decreasing rice, vegetables, fruit, bread and pasta – Increasing livestock and other animal products	• A shift from the current diet to alternative diets increases both the economic output and the environmental pressure. • Compared to the Mediterranean dietary pattern, shifting Iranian dietary patterns toward WHO and WCRF has a greater positive effect on economic output, but a more negative impact on the environment.
Eini-Zinab and Sobhani (14)	To compare the sustainability of traditional and local foods in Iran with Western Foods	Three traditional Iranian cusines (*Ashe Reshteh, Mirza- Ghassemi* and Tbrizi Koofteh) and three western foods (Pizza, Beef-estroganove and Pasta)	• Carbon FP • Water FP	Increase consumption of traditional and local Iranian foods/cuisines	• Food with high contribution of animal products had the highest carbon footprint. • Traditional and local Iranian foods seem to be more sustainable with low environmental effects compared to the selected western foods. • Traditional food patterns could be promoted through food and nutrition policy to achieve sustainable food and nutrition systems.
Sobhani et. al. (15)	To assess the compliance of the Iran's National Nutrition and Food Security Policy (INNFSP) with the components of the sustainable diets framework	National policy	–		• The compliance of the INNFSP with the components of a sustainable diet, without weighting importance and adequacy, was about 60.32%. The score was 60.69% when importance was weighted. • The percentage of compliance with the components of a sustainable diet was 41.79% when both importance and adequacy weighted. • In order to achieve a sustainable diet; which in addition to providing nutritional needs, has environmental, cultural, and economic sustainability; national food and nutrition policies needs to consider dimensions other than nutrition and health, as well.
Kalvani et. al. (16)	To evaluate and analyze water footprint of 14 important crops in Tehran province and to assess their water savings and losses in 2008–2014.	Apple, barley, bean, grapes, maize, onions, oranges, potatoes, rice, tomato, wheat, cherry, pear, peach	Water FP	Decrease wheat/wheat products	• Wheat and rice had the largest per capita water footprint • The consumption of wheat in Iran is high (2 times larger than global average). • It is recommended to reduce the consumption of wheat in Tehran or replace it with other crops.

(Continued)

TABLE 1 | Continued

First author (year)	Aim	Food item(s)/diet	Sustainable diet dimension(s) measured	Suggested change(s) in diet	Finding(s)
Soltani et. al. (17)	To investigate the role of current diets and types of modified diets on the need for environmental resources such as water, land, and inputs, including nutrients, energy, and greenhouse gas emissions.	Total diet	• Water FP • Carbon FP • Fertilizers use • Cost • Energy • Nutritional recommendation	Decrease rice (67%), potato (54%), oil (30%), sugar (53%), red meat (50%), chicken (55%), egg (48%), fruit (6%), and Increase wheat flour (43%), legumes (78%), and vegetables (32%)	• Plant-based diet can reduce the need for blue water resources by 30%, fertilizer by 8–12%, energy by 15%, and greenhouse gas emissions by 10-14%. The cost of these diets are also 20–24% less; • Implementing and adopting sustainable plant-based diets requires cultural and educational efforts.
Sobhani et. al. (18)	To assess different scenarios to reduce water use by following healthy diet recommendations/ to suggest a healthy diet with low water FP for the Urmia population.	Total diet in Urmia based on a FFQ	• Water FP • Nutritional value • Cultural acceptance	Decrease "bread, cereal, rice, and pasta" (14%) and meats (3%) Increase dairy (14%) and fruits (6%)	A healthy diet with greater proportion of energy from fruits, and lower ratio from "bread, cereal, rice, and pasta", and substitution of meats with beans can supply all recommended dietary allowances while reducing water use by 49%.
Mirzaie-Nodoushan et. al. (19)	To investigate the effects of diet change on reducing water consumption in Iran, while meeting its nutrition requirements.	Iranian food basket	• Water FP • Nutritional recommendations • Cultural acceptance	Decreases red meat (47%), fruits (35–44%), poultry (9–42%), vegetable oil (13–25%), sugar (26–30%), and rice (17–40%). Increase vegetables (80%), milk (78–80%), pulses (51–75%), fish (29–80%), eggs (37–79%), and wheat (15–21%)	These changes resulted in 7.9%-16.7% decrease in water footprint.
Eini-Zinab et. al. (20)	To develope a healthy, low-cost and environmental-friendly food basket for Iran, based on current consumption	Total diet	• Water FP • Carbon FP • Cost • Nutritional recommendation • Cultural acceptance	Decrease the "bread, cereal, rice, and pasta" (34%), "meat, poultry, fish, eggs, legumes, and nuts" (11%) and "fats, oils, sugars, and sweets" (24%) Increase dairy (34%), fruits (26%) and vegetable (8%) groups and cereals (38%), poultry (45%) and vegetable oil (30%) subgroups	In the sustainable diet model extracted, there was a 14% reduction in total water footprint, a 14% decrease in total carbon footprint, and a 23% decrease in the cost, as well as 7% increase in NRF of diet compared with the usual consumption.
Edalati et. al. (21)	To analyze a canteen menu of the School of Nutrition Sciences and Food Technology sustainability and to develop a sustainable lunch menu	A campus lunch menu	• Water FP • Carbon FP • Land FP • Cost • Nutritional recommendation • Cultural acceptance	Decrease red meat Increase chicken or fish and vegetables	Replacing the sustainable food menu designed for Menu 1(rice-based) could decrease carbon, total water and land footprints and costs by 10, 13, 22 and 6%, respectively, and increased the NRF profile by 8%. Replacing the sustainable menu designed for Menu 2 (vegetable or meat-based, no rice but with wheat bread) could result in 25, 23, 27 and 28% decreases in carbon, total water and land footprints and costs,

(Continued)

TABLE 1 | Continued

First author (year)	Aim	Food item(s)/diet	Sustainable diet dimension(s) measured	Suggested change(s) in diet	Finding(s)
					respectively, and increased the NRF profile by 23%.
Noormohammadi et.al. (22)	To suggest dietary scenarios for decreasing GHG emissions	Total diet	• Carbon FP • Nutrition recommendation • Cultural acceptance	Decrease red and white meats, eggs, grains, fats and oils, and sweets Increase vegetables, fruits, legumes, nuts, and dairy.	• A healthy diet with a higher proportion of vegetables, fruits, legumes, nuts, and dairy, and a lower share of red and white meats, eggs, grains, fats and oils, and sweets can reduce carbon FP by %50.
Sobhani et. al. (23)	To evaluate the sustainability of Iranian FBDG in comparison with the usual diet and with the selected food pyramids.	Total diet	• Water FP • Carbon FP • Land FP • Cost • Nutritional recommendation • Cultural acceptance	Increase legumes and nuts	• Replacing the usual dietary intake of the Iranians with an optimal diet based on the 2016 Iranian FBDG was associated with reductions equal to 20.9 % for water footprint, 22.48 % for carbon footprint, 20.39 % for land footprint, 31.83 % for cost, as well as 7.64 % increase in NRF index.

FP, footprint.

criteria (17, 20, 21, 23). Some details of the findings are as follows:

Noormohammadi et al., using linear programming, showed that compared to the usual intake (in Urmia city, in north-west Iran), a healthy diet with a higher proportion of vegetables (20%), fruits (25%), legumes (7%), nuts (45%), and dairy (115%) and a lower share of red and white meats (92%), eggs (70%), grains (42%), fats and oils, and sweets (12%) can lead to about 50% decrease in carbon footprint. The recommended diet was nutritionally adequate, although its cost was not measured (22). Rahman et al. (13), using food balance sheet data of Iran, showed that a shift from the current diet to alternative diets, based on the WHO, the World Cancer Research Fund (WCRF), or the Mediterranean diet recommendations, will require to decrease the amount of rice, vegetables, fruits, bread, and pasta and increase comsumption of animal products. They showed that such changes will result in positive effects on economic output, while it has a negative environmental impact due to increasing carbon footprint (13). On the other hand, studies that considered carbon footprint in addition to other dimensions of the sustainable diet have shown that diets with higher consumption of vegetables, legumes, and cereals and lower contribution of red meat, rice, sugar, and egg can reduce greenhouse gas emissions by 10–14% as well as cost (20–28%) compared to the usual diet (14, 17, 20, 21).

With regard to minimizing water footprint of diet, a study by Sobhani et al., using linear programming, showed that including a greater proportion of energy from fruits (6%) and dairy (14%) and decreasing the energy share of bread, cereal, rice, and pasta (14%) as well as meat, fish, poultry, and eggs (3%) can supply all the recommended dietary allowances while reducing water use by 49% (18). Although cultural acceptance of dietary change was considered through minimizing the distance between an optimal diet with the usual diet, other environmental indicators (e.g., carbon or land footprint) or costs were not calculated. Similarly, in a study by Mirzaie-Nodoushan et al. (19), the minimized total water footprint scenario of 7.9 to 16.7% can be resulted by increasing intakes of vegetables (80%), milk (78–80%), pulses (51–75%), fish (29–80%), eggs (37–79%), and wheat (15–21%) and decreasing red meat (47%), fruits (35–44%), poultry (9–42%), vegetable oil (13–25%), sugar (26–30%), and rice (17–40%). Another study that included carbon footprint and cost in addition to water footprint, nutrition recommendation, and cultural acceptance reported a 14% reduction in total water footprint (20). Soltani et al. showed that 30% reduction in blue water footprint, i.e., surface and groundwater during the production of a product and through its supply chain can occur by following a plant-based diet (with lower rice, potato, oil, sugar, red meat, chicken, egg, and higher amount of wheat flour, legumes, and vegetables consumption compared to the usual diet) (17).

On the other hand, Edalati et al., in an attempt to design a sustainable lunch menu for a university campus in Tehran, showed that compared with the usual menu, including a higher amount of bread and vegetables while decreasing rice and red meat in the menu, it leads to 23% decrease in water footprint (21). Further, it has been shown that a higher share of bread and vegetables in traditional and local Iranian foods lead to a lower water footprint compared with western foods (14). While most studies have recommended reducing rice consumption (18, 19), Kalvani et al., analysis of water footprint of 14 important crops in Tehran province, recommended reducing the consumption of wheat, which is mainly consumed as white flat bread and suggested replacing it with other crops in order to reduce water FP (16).

A total of four studies simultaneously considered dimensions of sustainable diet including nutritional, environmental, cost, and cultural acceptability in their analysis (17, 20, 21, 23). Despite the differences in findings, almost all emphasized on higher intake of dairy (17, 20), vegetables (17, 20, 21), cereals (17, 20, 21), fruits (20), and legumes (17, 21, 23) and in the meantime, lower intake of meat (17, 20, 21), rice (17, 20, 21), sugar (17, 20, 21), fats (17, 20, 21), and egg (17, 20, 21) in order to have a sustainable diet. With respect to poultry consumption, there was inconsistency in the results; one study recommended higher consumption (20), while the other study emphasized reducing it (17) in order to have a more sustainable diet in Iran.

In a study by Sobhani et al., aiming at developing a healthy, low-cost, and environmentally-friendly food basket, an optimal food basket compared with the usual consumption, resulted in 14% reduction in both the total water footprint as well as total carbon footprint, 23% decrease in the cost, and 7% increase in nutrient rich foods (NRF) of diet (20). Soltani et al. suggested that a plant-based diet can reduce the need for blue water resources by 30%, fertilizer by 8–12%, energy by 15%, and greenhouse gas emissions by 10–14%. Also, the diet cost 20–24% less (17). Cultural acceptance was not considered in the latter study and this may explain the difference between its findings with those of Sobhani et al. (20). Also, the sustainable lunch menu proposed by Edalati et al. included more dishes based on chicken or fish with vegetables and fewer red meat and rice-based dishes. They showed that this change can result in decreasing carbon, total water, and land footprints as well as costs of the lunch menu by 25, 23, 27, and 28%, respectively, and 23% increase in its NRF profile (21).

Finally, evaluating the sustainability of the first (2005) (24) and second (2016) (25) versions of national Food-Based Dietary Guidelines (FBDGs) in Iran in comparison with the usual diet showed that while both the FBDGs have lower cost, water and carbon footprint, and higher nutritional value, the difference was statistically significant only for the more recent version. The second national FBDG emphasizes legumes and nuts by defining them as a separate food group from the meat group. Replacing the usual dietary intake of the Iranians with the optimal diet based on the last Iranian FBDG (2016) was associated with reductions equal to 20.9% for water footprint, 22.48% for carbon footprint, 20.39% for land footprint, 31.83% for cost, and 7.64% increase in NRF index (23).

Considering Sustainable Diet in Food and Nutrition Policies in Iran

No direct address to "sustainable diet" in the food and nutrition policy documents in Iran was found. However, a recent analysis on the National Food and Nutrition Security Policy compliance in Iran with the components of a sustainable diet reported a score of 60% (15). Through the mentioned policy document, some of the strategies needed to achieve a sustainable diet have been recommended including use of effective economic tools to promote healthy nutrition (e.g., sin tax, subsidies, and loans), obligatory and optional nutrient fortification of the main and complementary foods, healthy formulation of produced foods, promoting home vegetable gardens in rural areas, and distribution and supply of vegetables, fruits, and legumes in remote areas of the country through rural cooperatives to increase access of community to micronutrient resources and finally redirecting food subsidies to increase consumption of vegetables, fruits, meat, milk, and dairy products (26). Similar recommendation has been also provided by the National Action Plan for Prevention and Control of NCDs in Iran aiming at 30% reduction in sodium/salt intake and zero amount of trans-fatty acids in oils and food products by 2025 (27). However, cost and ecological aspects of diet were not taken into account in both the documents.

DISCUSSION

"Sustainable diets" are not mentioned directly and clearly in the SDGs; however, according to the post-2020 global biodiversity framework, in achieving the vision of "living in harmony with nature," shifting toward a sustainable diet is one of the key actions required (28). This study is an attempt undertaken to summarize current literature on the sustainability of the Iranian diet in order to inform decision-makers on changes required to dietary guidelines and policies to be both nutritionally adequate and environmentally sustainable and to identify potential trade-offs.

In Iran, given the fact that there is low intake of vegetables, fruits, and dairy (29), a large share of food in the household budget (about 24%) (30) and an increasing trend of water shortage (31), CO_2 emission (32), and warmer climate (33) during the recent years, moving toward a sustainable diet, is inevitable. The findings of this study showed that an increase in dairy, fruits, vegetables, cereals, poultry, and legumes consumption and a decrease in intake of bread, rice and pasta, red meat, eggs, fats, sugar, and sweets are the main changes being suggested in the Iranian diet in order to move it toward sustainablility. These findings are in line with the EAT-Lancet recommendation that emphasizes a diet rich in plant-based foods with fewer animal sources in order to both improve the health and benefit of the environment (4). The specific recommendations identified with respect to the required changes in the Iranian diet are as follows:

First, the need for consuming higher amounts of varied fruits and vegetables as a step toward a sustainable diet was reconfirmed in this study. Sustainable diets are characterized by varied and high amounts of vegetables and fruits (34), which make them more nutritious and environmentally friendly, with a lower risk of chronic diseases compared to other dietary patterns (35). Vieux et al. investigating changes needed to improve sustainability of the diet across Europe and concluded that this can be achieved by substituting food items from the sugar/fat/alcohol group with fruits, vegetables, and starches in "the diet" (36). It is worth noting that consumption of fruit and vegetables in Iran has been consistently reported to be lower than minimum recommended amount of 400 g per capita per day by the WHO (29, 37). Lack of access to fruits and vegetables in all the regions and in all the seasons of the year, high price of fruits in particular, and the high loss and waste rate of these products are the identified reasons behind insufficient intake of these food groups in Iran (38).

Second, reducing red meat consumption and substituting it with legumes (plant sources of proteins) or poultry in the Iranian diet is another recommendation that has been raised by the studies on sustainable diet in Iran. Since 1960s, consumption of animal-based foods has increased throughout the world due to rising global average income, higher standards of living, and increased efficiency of production (35). Similarly, the share of meat in the Iranian food basket has increased over the past 30 years; although in the recent years, due to the economic crises of country, the increase rate has slowed down (29). Bahn et al. had previously proposed that reducing red meat consumption and simultaneously increasing consumption of vegetables/beans in Middle East and North Africa (MENA) countries can result in positive environmental effects (39). Considering higher water and carbon footprint, energy input, fertilizer, and pesticide use with an animal-based diet compared with a plant-based diet (35), low-to-moderate consumption of seafood and poultry and zero to low consumption of red meat and processed meat are recommended in order to have a sustainable diet (4). With lower water Footprint (FP) and price of poultry compared to red meat, it seems poultry is a more realistic choice (40, 41). Fish is anoter alternative, which is not much emphasized in Iranian population due to its higher price and low desirability in the majority of Iranians (it is highly desirable in North and South of Iran). Average consumption of fish in the Iranian diet (8 g/day) (29) is way below the recommended amount of 30 g/day (42).

Another suitable alternative to red meat is legumes. Legumes are inexpensive and sustainable sources of protein with low carbon and water FP that are part of the traditional diets in most cultures including Iran (43). Based on a study by Roos et al., reducing daily per capita intake of meat from 110 to 55 g and replacing it with 50 g of beans is associated with a 20% reduction in environmental footprint (44). Besides, legumes production positively affects soil quality, biodiversity, and enriches the soil through nitrogen fixation (45). The role of legumes in achieving a sustainable diet is so important that the FAO designated 2016 as the year of pulses as a subgroup of legumes and introduced it as food crops that can play a major role in addressing future global food security and environmental challenges as well as contributing to healthy diets (46). For these reasons, in the second version of FBDG in Iran (2016), legumes and nuts have been defined as a separate food group from the meat group. Comparing the sustainability dimensions of the first and second FBDGs of the country showed that the recent version can result in a diet with lower cost, water, and carbon footprint, provides higher nutritional value, and can be more in line with a sustainable diet compared to the previous version (2005) (23).

Third, replacing refined carbohydrates (e.g., white bread, polished/white rice) with whole-grain products is another required step identified to move toward a sustainable diet in Iran. White rice and white flat bread are the main staple foods in the Iranian diet, the share of the latter being higher among those with lower income (47). Iran is the 13th biggest white rice consumer worldwide (47) with an annual consumption of 36.6 kg per capita, which is 7 times more than the European Union (5.3 kg per capita) (48). Higher white rice and bread intake are associated with an increased risk of type 2 diabetes (49, 50). Besides, wheat and rice have the largest per capita water FP in the country (16). It should be noted that water FP varies for each crop depending on irrigation and on average rice cultivation has the least water efficiency (51). Studies on the sustainable diet in China also showed that a decrease in rice consumption is required due to its role as the largest contributor to reductions in GHG emissions (52). Promotion of less refined and polished rice or wheat will require policy change at the production level as well as consumer education.

The fourth recommendation identified through this study in order to have a more sustainable diet is decreasing sugar and fats. Consumption of oils and sugar by Iranian is 20 and 38% of daily energy intake, respectively, which is higher than the recommended amounts (53). Since 1960s, due to the improvement of vegetable oil production technology and increased supply, its price has decreased worldwide (54). Also, the increased supply of processed foods and sweetened beverages has resulted in higher intake of sugars (55). Despite low environmental impact of sugar and fat consumption, considering their negative impact on health and prevention of NCDs (37), the need for reducing their intake to 31 g/day for added sugar and 40 g/day for added fats has been emphasized (4).

The final recommendation is the only one that seems differerent with the EAT-Lancet recommendation of decreasing dairy products; however, due to very low consumption of dairy products in Iran (168 g/day) (27), there is a need to increase the consumption of this food group in order to comply with the recommendation of 250 g/day (29). In fact, the average consumption level of dairy products in Iran is less than half the recommended level and has had a decreasing trend due to a drastic rise in the price over the last decade as a result of inflation and poor governmental support policies (56). Similarly, Donati et al. have recommended increasing dairy as part of the changes required to achieve a more sustainable diet in adolescents in Italy (57).

Based on this study, there is no clear and direct reference to sustainable diet in the related documents in Iran and there is no consistent approach with respect to the policies and strategies to move toward a sustainable food and nutrition system in policy documents in Iran. In one hand, the food production policies of country have been more focused on increasing agricultural production aiming at self-sufficiency and sustainable agriculture with less attention to the quality and sustainability of the food consumed, i.e., sustainable diet (58). On the other hand, due to the high prevalence of NCDs, the health sector has properly focused on health aspect of a sustainable diet through investment on policies and strategies to decrease the amount of sugar, salt, and fat (specifically trans-fatty acids) in the Iranian diet. The National Action Plan for Prevention and Control of NCDs in Iran has strongly focused on reducing sodium/salt intake as well as saturated and trans-fatty acids in oils by 2025 (27). In line with these policies, several regulatory policies/programs have been

TABLE 2 | Regional recommended strategies for a sustainable food diet, current status of related policies in Iran, and recommended actions.

"Game changing" food systems actions (68)	Recommended priority actions 2020–2030	Existing policies in Iran	Gap/recommended policies for Iran
Fiscal policies for healthy and sustainable diets.	• Implement a tax on sugar-sweetened beverages and use other taxes and subsidies to promote healthy diets • Review food subsidy programs and progressively eliminate subsidies for all types of fats/oils and sugar.	• 2015: A maximum tax of 10% was imposed to unhealthy food (63). • The Iranian budget law for the fiscal year 2013–2014 obligated the government to taxation of soft drink at rate of 15 and 20% for locally produced and imported goods, respectively (63). • 2010: Subsidy on sugar and vegetable oil were reduced (69). • 2014: Eliminating subsidies for milk. (70)	Elimination of subsidies on vegetable oil and sugar and instead shifting subsidies to healthier foodstuffs (i.e, fruits, vegetables and dairy) (71).
Public food procurement and service policies for a healthy diet sustainably produced	• Introduce and enforce mandatory guidelines for provision of healthy food in public institutions (e.g., schools, hospitals, military, prison and other government institutions).	• 2005: The National FBDGs was developed and introduces as one of the main tools to raise awareness regarding healthy diet. Second version of the FBDG was introduced in 2016 (24).	Reevaluating the national FBDG, as well as the thrift food basket in terms of food grouping and recommendations in order to make it more sustainable.
Regulation of marketing of foods and non-alcoholic beverages, including breastmilk substitutes	• Implement the WHO Set of recommendations on marketing of foods and non-alcoholic beverages to children • Reinforce the package of policies and interventions to promote, protect and support breastfeeding and appropriate complementary feeding.	• 1995: Code of marketing of the breast milk substitutes was initiated in the country (72). • The 5th national develoment plan (2011–2016), bans advertising unhealthy goods and services to the public (63). • 2014: Healthy school canteen program was initiated to regulate access to healthy foods in schools (73). • 1978: Food advertisements in school and educational facilities are banned (74).	"Water penalty" and the "water tax" for food products with high water footprint (75). Eliminate legal barriers and disproportionate food-safety standards that lead to high waste rates (71). Investments in water-friendly food processing technologies to tackle natural resources overexploitation and promote corporate profitability (75).
Food products reformulation	• Progressively reduce intakes of salt, sugars and saturated fats by improving the nutritional quality of foods through government-led reformulation programmes. • Eliminate trans fats through the development of legislation to ban the use of industrially-produced trans fats in the food chain	• 2015: Mandatory upper limit of salt in most commonly consumed canned foods e.g., tomato paste and salty snacks, and all types of bread was decreased (1.8%) (59). Also, the standard of salt in cheese was decreased from 4 to 3% and in dough (fermented yogurt drink) was decreased from 1 to 0.8% (59). • 2016–2017: The standard of salt in bread was further decreased to 1% (59). • 2017: The standard of SFA in edible oil decreased to <25 (59). • 2018: Salt use in probiotic yogurts was banned (59). • Food reformulation initiatives for reducing the amount of sugar, salt, and fat, specifically the industrially-produced trans-fatty acids in industrial food products (61, 62).	Decrasing flour milling degree to incease fiber and nutrient content of flour and bread (76). Focus on some of the new technologies being developed to create alternatives to animal products, such as meat, milk and egg, which should reduce the amount of meat consumed. Also, increasing number of fiber-containing products is highly recommended (77, 78).
Front-of-pack labeling	• Implement mandatory standards for ingredient listing, back-of-pack nutrient declarations and simplified front-of-pack labeling for all pre-packaged foods	• 2014: MOHME launched Front-of-Pack nutrition labeling policy (as traffic light scheme) with the objective of reducing sodium, trans fatty acid and sugar intake in accordance with the national action plan for control and prevention of NCDs (63).	Food labels including information about links between food and climate chang are effective in encouraging sustainable food choices (79)
Food fortification	• Implement and monitor policies and practices for wheat flour fortification and for salt iodization, in line with the latest WHO recommendations on best practice.	• Mandatory flour fortification with iron and folic acid was implemented in one province from 2001 and expanded simultaneously to the other provinces in Iran (80). • since 2000, Iran has been recognized by WHO-as an IDD free nation through integration of IDD control into the health network and mandatory iodization of household salt (81). • Voluntary fortification of edible oil, milk, cakes, and pasta with vitamin D (82).	Universal fortification along with small dietary shifts represents an approach to improve the vitamin D status of the general population, at a high acceptability without affecting the carbon footprint (83)

implemented to tackle NCDs, e.g., setting a mandatory upper limit of salt in bread (59, 60), food reformulation initiatives for reducing the amount of sugar, salt, and fat, specifically the industrially-produced trans-fatty acids in industrial food products (61, 62), sin tax policy on increasing the price of sugar-sweetened beverages (SSB) (63), traffic light nutrition labeling for more than 80% of the manufactured food products (64), and educational interventions/campaigns to improve awareness and food choices of consumers (65). In addition, the national FBDG, as one of the main tools to raise awareness with respect to appropriate and healthy diet, has been in place since 2005 (24). However, within all these policies, there is a gap in adressing ecological aspects of a sustainable diet (e.g., water, land, and carbon footprints) with a specific reference to context of Iran. Recently, there have been attempts to evaluate all the dimensions of sustainablility of Iran FBDG (23) to pave the ground for a sustainable FBDG in the close future.

One other aspect that is almost ignored in study with respect to sustainable diet in Iran is measurement and interventions with respect to food loss and waste. There are different estimations of the amount of waste and loss of agricultural products in Iran, ranging from 18.5 up to about 35% (66). A report by the Food Producers Cooperatives of Iran indicates that out of about 130 million tons of foods being produces in Iran, 25 million tons are lost (67). According to the study conducted by Fami et al. in Tehran, every consumer wastes about 27 kg of edible food annually (66). A total of seven food items identified with the highest amount of waste at household level are bread, cooked rice, cooked pasta, fresh fruits, fresh vegetables, salads, milk, and dairy products (66). Developement of proper infrastructure and skills to decrease the loss at the production and distribution levels as well as improving knowledge and skills on planning, preparation, and storage, and raising awareness about values and consequences of avoiding food waste at the procurement and consumption levels (66) can lead to decrease food loss and food waste.

Table 2 presents the current policies across the food system that support move toward a sustainable diet in Iran, based on the available recommendations (68) as well as gaps and recommended policies to improve current efforts.

To the best of our knowledge, this study is the first study on current literature on the sustainability of the Iranian diet and recommended changes. The findings can serve to guide policymakers and planners of the food and nutrition food system to develop and implement policies to shift to a sustainable dietary pattern in Iranian population. This study also has several limitations that need to be considered in evaluating its findings. The quality of the studies included in this study was not assessed. Thus, the conclusions are based on the existence of studies rather than their intrinsic quality. Moreover, the Iranian food intake values based on a nutritional assessment were not available for comparison with the recommended values.

CONCLUSION

This study showed that there is an urgent need to accelerate the move toward a sustainable diet in Iran. For this purpose, increasing consumption of dairy, fruits, vegetables, cereals, poultry, and legumes and decreasing bread, rice and pasta, red meat, eggs, hydrogenated fats, sugar, and sweets intake are the main changes suggested. Development of an enabling environment to promote moving toward a sustainable diet will require policy and action to: (1) Improve public awareness and interest; (2) Support study to provide evidence and identify possible alternatives; and (3) Plan and implement interventions/programs to promote and facilitate healthy and sustainable diets. In this regard, defining scientific targets to achieve sustainable diet and food production and re-evaluating existing policies related to food and nutrition system are prerequisites to such transformation.

AUTHOR CONTRIBUTIONS

NO and AA contribute to the conceptualization. SS and NO contribute to the data collection, reviewing the studies, analyses, and writing—original draft preparation. NO, ZA, and AA contribute to the writing—review and editing. All the authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Popkin BM. Technology, transport, globalization and the nutrition transition food policy. *Food Policy.* (2006) 31:554–69. doi: 10.1016/j.foodpol.2006.02.008
2. Kearney J. Food consumption trends and drivers. *Philos Trans R Soc Lond B Biol Sci.* (2010) 365:2793–807. doi: 10.1098/rstb.2010.0149
3. Searchinger T, Waite R, Hanson C, Ranganathan J, Dumas P, Matthews E, et al. *Creating a sustainable food future: a menu of solutions to feed nearly 10 billion people by 2050. Final report.* Washington, D.C.:WRI (2019).
4. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4
5. Gold K, McBurney RP. Conservation of plant diversity for sustainable diets. In: *Sustainable Diets and Biodiversity–Directions and Solutions for Policy, Research and Action.* Rome: FAO Headquarters (2012).
6. Tokunaga M, Takahashi T, B Singh R, Rupini D, Toda E, Nakamura T, et al. Diet, nutrients and noncommunicable diseases. *Open Nutraceuticals J.* (2012) 5:146–59. doi: 10.2174/1876396001205010146
7. Krishnaswamy K, Gayathri R. Nature's bountiful gift to humankind: vegetables & fruits & their role in cardiovascular disease & diabetes. *Indian J Med Res.* (2018) 148:569. doi: 10.4103/ijmr.IJMR_1780_18
8. Aleksandrowicz L, Green R, Joy E, Smith P, Haines A. The impacts of adopting environmentally sustainable and healthy diets on greenhouse gas emissions, land use, and water use: a systematic review. *PLoS ONE.* (2016) 11:e0165797. doi: 10.1371/journal.pone.0165797

9. Perignon M, Vieux F, Soler L-G, Masset G, Darmon N. Improving diet sustainability through evolution of food choices: review of epidemiological studies on the environmental impact of diets. *Nutr Rev.* (2017) 75:2–17. doi: 10.1093/nutrit/nuw043
10. Misra AK. Climate change and challenges of water and food security. *Int J Sustain Built Environ.* (2014) 3:153–65. doi: 10.1016/j.ijsbe.2014.04.006
11. Kelishadi R, Alikhani S, Delavari A, Alaedini F, Safaie A, Hojatzadeh E. Obesity and associated lifestyle behaviours in Iran: findings from the first national non-communicable disease risk factor surveillance survey. *Public Health Nutr.* (2008) 11:246–51. doi: 10.1017/S1368980007000262
12. World Health Organization. *Sustainable Healthy Diets: Guiding Principles*. Rome: Food & Agriculture Org (2019).
13. Rahmani R, Bakhshoodeh M, Zibaei M, Heijman W, Eftekhari MH. Economic and environmental impacts of dietary changes in Iran: an input-output analysis. *Int J Food Syst Dyn.* (2011) 2:447–63. doi: 10.18461/ijfsd.v2i4.248
14. Eini-Zinab H, Sobhani R. Sustainable diets and traditional local foods. *Iran J Nutr Sci Food Technol.* (2017) 12:S151–8. Available online at: https://nsft.sbmu.ac.ir/article-1-2630-fa.pdf (accessed June, 2021).
15. Sobhani S, Sheikhi M, Eini-Zinab H, Mohammadi-Nasrabadi F. Compliance of Iran's national nutrition and food security policy (2012-2020) with components of sustainable diets framework. *Iran J Nutr Sci Food Technol.* (2018) 13:153–60.
16. Kalvani SR, Sharaai A, Manaf L, Hamidian A. Evaluation of water footprint of selected crop consumption in Tehran Province. *Appl Ecol Environ Research.* (2019) 17:11033–44. doi: 10.21837/pm.v17i10.634
17. Soltani E, Soltani A, Alimagham M, Eskandar Z. *Diet and its Role in the Necessary Resources for Agricultural Production*. Gorgan: Gorgan University of Agricultural Sciences and Natural Resources (2019).
18. Sobhani SR, Rezazadeh A, Omidvar N, Eini-Zinab H. Healthy diet: a step toward a sustainable diet by reducing water footprint. *J Sci Food Agric.* (2019) 99:3769–75. doi: 10.1002/jsfa.9591
19. Mirzaie-Nodoushan F, Morid S, Dehghanisanij H. Reducing water footprints through healthy and reasonable changes in diet and imported products. *Sustain Prod Consumption.* (2020) 23:30–41. doi: 10.1016/j.spc.2020.04.002
20. Eini-Zinab H, Sobhani SR, Rezazadeh A. Designing a healthy, low-cost and environmentally sustainable food basket: an optimisation study. *Public Health Nutr.* (2021) 24:1952–61. doi: 10.1017/S1368980020003729
21. Edalati S. .Sobhani R, Fallah F, Renani MM, Tavakoli S, .Nazari H, et al. Analysis of a campus lunch menu for aspects of sustainable diets and designing a sustainable lunch menu. *Iran J Nutr Sci Food Technol.* (2021) 16:37–46. doi: 10.52547/nsft.16.1.37
22. Noormohammadi M, Eini-Zinab H, Rezazadeh A, Omidvar N, Sobhani SR. A step toward a sustainable diet by reducing carbon footprint: a case study in Iran. *J Environ Health Sustain Develop.* (2021) 6:1423–32.
23. Sobhani SR, Edalati S, Eini-Zinab H, Kennedy G, Omidvar N, A. comparative analysis of sustainability of the usual food intakes of the Iranian population, Iranian food based dietary guidelines and optimized dietary models. *unpublished.* (2021).
24. Montagnese C, Santarpia L, Iavarone F, Strangio F, Sangiovanni B, Buonifacio M, et al. Food-based dietary guidelines around the world: eastern Mediterranean and Middle Eastern countries. *Nutrients.* (2019) 11:1325. doi: 10.3390/nu11061325
25. Safavi S, Omidvar N, Djazayery A, Minaie M, Hooshiarrad A, Sheikoleslam R. Development of food-based dietary guidelines for Iran: a preliminary report. *Ann Nutr Metab*. (2007) 51:32–5. doi: 10.1159/000103565
26. Damari B, Abdollahi Z, Hajifaraji M, Rezazadeh A. Nutrition and food security policy in the Islamic Republic of Iran: situation analysis and roadmap towards 2021. *East Mediterr Health J.* (2018) 24:177–88. doi: 10.26719/2018.24.2.177
27. Ghazizadeh-Hashemi S, Larijani B. *National Action Plan for Prevention and Control of Non Communicable Diseases and the Related Risk Factors in the Islamic Republic of Iran, 2015–2025*. Tehran, Iran: Aftab e Andisheh Publications (2015). p. 47–65.
28. Delabre I, Rodriguez LO, Smallwood JM, Scharlemann JP, Alcamo J, Antonarakis AS, et al. Actions on sustainable food production and consumption for the post-2020 global biodiversity framework. *Sci Adv*. (2021) 7:eabc8259. doi: 10.1126/sciadv.abc8259
29. Sobhani SR, Eini-Zinab H, Rezazadeh A. Assessing the changes in Iranian household food basket using national household budget and expenditure survey data, 1991–2017. *Int J Prev Med*. (2021) 12:148–57. doi: 10.4103/ijpvm.IJPVM_404_19
30. Central Bank of the Islamic Republic of Iran. *The Household Budget Survey in Urban Area.* Central Bank of the Islamic Republic of Iran (2016) 1:8–10.
31. Zarghami M, Abdi A, Babaeian I, Hassanzadeh Y, Kanani R. Impacts of climate change on runoffs in East Azerbaijan, Iran. *Glob Planet Change.* (2011) 78:137–46. doi: 10.1016/j.gloplacha.2011.06.003
32. Lotfalipour MR, Falahi MA, Ashena M. Economic growth, CO2 emissions, and fossil fuels consumption in Iran. *Energy.* (2010) 35:5115–20. doi: 10.1016/j.energy.2010.08.004
33. Tabari H, Somee BS, Zadeh MR. Testing for long-term trends in climatic variables in Iran. *Atmos Res.* (2011) 100:132–40. doi: 10.1016/j.atmosres.2011.01.005
34. *Shifting to Sustainable Diets: United Nations*. Available from: https://www.un.org/en/academic-impact/shifting-sustainable-diets
35. Reynolds CJ, Buckley JD, Weinstein P, Boland J. Are the dietary guidelines for meat, fat, fruit and vegetable consumption appropriate for environmental sustainability? A review of the literature. *Nutrients.* (2014) 6:2251–65. doi: 10.3390/nu6062251
36. Vieux F, Perignon M, Gazan R, Darmon N. Dietary changes needed to improve diet sustainability: are they similar across Europe? *Eur J Clin Nutr.* (2018) 72:951–60. doi: 10.1038/s41430-017-0080-z
37. World Health Organization. *Diet, Nutrition, and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation*. Geneva: World Health Organization (2003).
38. Mason-D'Croz D, Bogard JR, Sulser TB, Cenacchi N, Dunston S, Herrero M, et al. Gaps between fruit and vegetable production, demand, and recommended consumption at global and national levels: an integrated modelling study. *Lancet Planet Health.* (2019) 3:e318–29. doi: 10.1016/S2542-5196(19)30095-6
39. Bahn R, Labban SE. Hwalla N. Impacts of shifting to healthier food consumption patterns on environmental sustainability in MENA countries. *Sustain Sci.* (2019) 14:1131–46. doi: 10.1007/s11625-018-0600-3
40. *Statistical Center of Iran Household, Expenditure and Income.* Available from: https://www.amar.org.ir/english/Metadata/Statistical-Survey/Household-Expenditure-and-Income (accessed June 2020)
41. Mekonnen MM, Hoekstra AY. A global assessment of the water footprint of farm animal products. *Ecosystems.* (2012) 15:401–15. doi: 10.1007/s10021-011-9517-8
42. Nesheim MC, Oria M, Yih PT. Dietary recommendations for fish consumption. In: *A Framework for Assessing Effects of the Food System.* US: National Academies Press. (2015).
43. Semba RD, Ramsing R, Rahman N, Kraemer K, Bloem MW. Legumes as a sustainable source of protein in human diets. *Global Food Security.* (2021) 28:100520. doi: 10.1016/j.gfs.2021.100520
44. Röös E, Carlsson G, Ferawati F, Hefni M, Stephan A, Tidåker P, et al. Less meat, more legumes: prospects and challenges in the transition toward sustainable diets in Sweden. *Renew Agri Food Syst.* (2020) 35:192–205. doi: 10.1017/S1742170518000443
45. Meena RS, Das A, Yadav GS, Lal R. *Legumes for Soil Health and Sustainable Management*. Germany: Springer (2018). doi: 10.1007/978-981-13-0253-4
46. Calles T. The international year of pulses: what are they and why are they important. *Agri Develop.* (2016) 26:40–2. Available online at: http://www.fao.org/3/a-bl797e.pdf (accessed June, 2021).
47. Golozar A, Khalili D, Etemadi A, Poustchi H, Fazeltabar A, Hosseini F, et al. White rice intake and incidence of type-2 diabetes: analysis of two prospective cohort studies from Iran. *BMC Public Health.* (2017) 17:1–11. doi: 10.1186/s12889-016-3999-4
48. Jifroudi S, Teimoury E, Barzinpour F. Designing and planning a rice supply chain: a case study for Iran farmlands. *Decis Sci Lett.* (2020) 9:163–80. doi: 10.5267/j.dsl.2020.1.001
49. Aune D, Norat T, Romundstad P, Vatten LJ. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies. *Eur J Epidemiol.* (2013) 28:845–58. doi: 10.1007/s10654-013-9852-5
50. Hu EA, Pan A, Malik V, Sun Q. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. *BMJ.* (2012) 344:e1454. doi: 10.1136/bmj.e1454

51. Friel S, Barosh LJ, Lawrence M. Towards healthy and sustainable food consumption: an Australian case study. *Public Health Nutr.* (2014) 17:1156–66. doi: 10.1017/S1368980013001523
52. Song G, Li M. Fullana-i-Palmer P, Williamson D, Wang Y. Dietary changes to mitigate climate change and benefit public health in China. *Sci Total Environ.* (2017) 577:289–98. doi: 10.1016/j.scitotenv.2016.10.184
53. Abdi F, Atarodi KZ, Mirmiran P, Esteki T. *Surveying Global and Iranian Food Consumption Patterns: A Review of the Literature* (2015).
54. Hawkes C. Uneven dietary development: linking the policies and processes of globalization with the nutrition transition, obesity and diet-related chronic diseases. *Global Health.* (2006) 2:1–18. doi: 10.1186/1744-8603-2-4
55. Wittekind A, Walton J. Worldwide trends in dietary sugars intake. *Nutr Res Rev.* (2014) 27:330–45. doi: 10.1017/S0954422414000237
56. Rahnama H, Rajabpour S. Factors for consumer choice of dairy products in Iran. *Appetite.* (2017) 111:46–55. doi: 10.1016/j.appet.2016.12.004
57. Donati M, Menozzi D, Zighetti C, Rosi A, Zinetti A, Scazzina F. Towards a sustainable diet combining economic, environmental and nutritional objectives. *Appetite.* (2016) 106:48–57. doi: 10.1016/j.appet.2016.02.151
58. Soltani A, Alimagham S, Nehbandani A, Torabi B, Zeinali E, Zand E, et al. Future food self-sufficiency in Iran: a model-based analysis. *Global Food Secur.* (2020) 24:100351. doi: 10.1016/j.gfs.2020.100351
59. Al-Jawaldeh A, Taktouk M, Chatila A, Naalbandian S, Al-Thani A-AM, Alkhalaf MM, et al. Salt reduction initiatives in the Eastern Mediterranean region and evaluation of progress towards the 2025 global target: a systematic review. *Nutrients.* (2021) 13:2676. doi: 10.3390/nu13082676
60. Al Jawaldeh A, Al-Khamaiseh M. Assessment of salt concentration in bread commonly consumed in the Eastern Mediterranean Region. *East Mediterr Health J.* (2018) 24:18–24. doi: 10.26719/2018.24.1.18
61. Amini M, Doustmohammadian A, Ranjbar M. Dietary Risk Reduction Projects in Industrial Foods in Iran. *SRPH Journal of Fundamental Sciences and Technology.* (2021) 3:1–4.
62. Schafer M, de Figueiredo MD, Iran S, Jaeger-Erben M, Silva ME, Lazaro JC, et al. Imitation, adaptation, or local emergency?—A cross-country comparison of social innovations for sustainable consumption in Brazil, Germany, and Iran. *J Cleaner Prod.* (2021) 284:124740. doi: 10.1016/j.jclepro.2020.124740
63. Zargaraan A, Dinarvand R, Hosseini H. Nutritional traffic light labeling and taxation on unhealthy food products in Iran: health policies to prevent non-communicable diseases. *Iran Red Crescent Med J.* (2017) 19:e57874. doi: 10.5812/ircmj.57874
64. Edalati S, Omidvar N, Haghighian Roudsari A, Ghodsi D, Zargaraan A. Development and implementation of nutrition labelling in Iran: A retrospective policy analysis. *Int J Health Plann Manage.* (2020) 35:e28–44. doi: 10.1002/hpm.2924
65. Abdollahi Z, Sayyari A-A, Olang B, Ziaodini H, Fallah H, Abasalti Z, et al. Effect of educational intervention on healthy lifestyle in Iranian children and adolescents: the IRAN-Ending Childhood Obesity (IRAN-ECHO) program. *J Nutr Sci Diet.* (2019) 5:32–9. doi: 10.18502/jnsd.v5i1.5234
66. Fami HS, Aramyan LH, Sijtsema SJ, Alambaigi A. Determinants of household food waste behavior in Tehran city: a structural model. *Resour Conserv Recycling.* (2019) 143:154–66. doi: 10.1016/j.resconrec.2018.12.033
67. FinancialTribune, 2017. *Iran's Annual Food Waste at 25m Tons*. Available from: https://nancialtribune.com/articles/economy-domestic-economy/70344/iran-s-annual-food-waste-at-25m-tons
68. Al-Jawaldeh A, McColl K. Shifting to healthy and sustainable consumption patterns in the Eastern Mediterranean Region. *Unpublished.* (2021).
69. Hosseini SS, Charvadeh MP, Salami H, Flora C. The impact of the targeted subsidies policy on household food security in urban areas in Iran. *Cities.* (2017) 63:110–7. doi: 10.1016/j.cities.2017.01.003
70. Mohammadi-Nasrabadi F, Omidvar N, Khoshfetrat MR, Kolahdooz F. *Assessment and Analysis of Food Market in the Recent Decade in Iran (from 2005 to 2014)*. Tehran, Iran: National Nutrition and Food Technology Research Institute (2019).
71. Reisch L, Eberle U, Lorek S. Sustainable food consumption: an overview of contemporary issues and policies. *Sustain Sci Pract Policy.* (2013) 9:7–25. doi: 10.1080/15487733.2013.11908111
72. Al Jawaldeh A, Sayed G. Implementation of the international code of marketing of breast-milk substitutes in the Eastern Mediterranean Region. *East Mediterr Health J.* (2018) 24:25–32. doi: 10.26719/2018.24.1.25
73. Omidvar N, Babashahi M, Abdollahi Z, Al-Jawaldeh A. Enabling food environment in kindergartens and schools in iran for promoting healthy diet: is it on the right track? *Int J Environ Res Public Health.* (2021) 18:4114. doi: 10.3390/ijerph18084114
74. The Islamic Revolution Assembly. *The Regulation on the Establishment and Monitoring of the Work and Activity of Advertising Centers*. Tehran, Iran: The Islamic Revolution Assembly (1978).
75. Tsolakis N, Srai JS, Aivazidou E. Blue water footprint management in a UK poultry supply chain under environmental regulatory constraints. *Sustainability.* (2018) 10:625. doi: 10.3390/su10030625
76. Miller DD, Welch RM. Food system strategies for preventing micronutrient malnutrition. *Food Policy.* (2013) 42:115–28. doi: 10.1016/j.foodpol.2013.06.008
77. McClements D. Future foods: Is it possible to design a healthier and more sustainable food supply? *Nutr Bull.* (2020) 45:341–54. doi: 10.1111/nbu.12457
78. Bingley C. The technological challenges of reformulating with different dietary fibres. *Nutr Bull.* (2020) 45:328–31. doi: 10.1111/nbu.12451
79. Abrahamse W. How to effectively encourage sustainable food choices: a mini-review of available evidence. *Front Psychol.* (2020) 11:1–9. doi: 10.3389/fpsyg.2020.589674
80. Abdollahi Z, Elmadfa I, Djazayery A, Golalipour M, Sadighi J, Salehi F, et al. Efficacy of flour fortification with folic acid in women of childbearing age in Iran. *Ann Nutr Metab.* (2011) 58:188–96. doi: 10.1159/000329726
81. Azizi F, Mehran L. Experiences in the prevention, control and elimination of iodine deficiency disorders: a regional perspective. *East Mediterr Health J.* (2004) 10:761–70. doi: 10.26719/2004.10.6.761
82. Ejtahed H-S, Shab-Bidar S, Hosseinpanah F, Mirmiran P, Azizi F. Estimation of vitamin D intake based on a scenario for fortification of dairy products with vitamin D in a Tehranian population, Iran. *J Am Coll Nutr.* (2016) 35:383–91. doi: 10.1080/07315724.2015.1022269
83. Bruins MJ, Létinois U. Adequate vitamin D Intake cannot be achieved within carbon emission limits unless food is fortified: a simulation study. *Nutrients.* (2021) 13:592. doi: 10.3390/nu13020592

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Assessment of the Methodology That Is Used to Determine the Nutritional Sustainability of the Mediterranean Diet—A Scoping Review

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Mediterranean diet (MedDiet) is often used as an example of a sustainable diet that promotes a sustainable food system. MedDiet presents low environmental impacts, is characterized by high sociocultural food values, allows for positive local economic returns, and presents major health and nutrition benefits. Previous studies have not systematically examined the methodological assessment of MedDiet nutritional sustainability. In our study, we review the methodological assessment of nutritional sustainability, filling a crucial gap in the literature that can inform the state of the art regarding the cross-disciplinary assessment of MedDiet nutritional sustainability. Through a systematic search on PubMed and Scopus, we identified 28 studies, published between 2013 and 2021, that dealt with the MedDiet nutritional sustainability. Studies that assessed the sustainability of MedDiet based on dietary consumption data, studies that explored the MedDiet sustainability resorting to dietary scenarios, and studies with a mixed approach (dietary consumption and dietary scenarios) and proposals of methodological approaches to assess the MedDiet nutritional sustainability were summarized. We identified 24 studies exploring the dimensions of nutritional sustainability of the MedDiet, and 4 proposing the methodological approaches to assess the MedDiet nutritional sustainability or the sustainability of MedDiet typical agro-foods. From the 24 studies exploring the sustainability of MedDiet, none fully addressed the complexity of the four dimensions of nutritional sustainability (environmental, economic, socio-cultural, and health-nutrition). One of the methodological proposals to assess the MedDiet nutritional sustainability contemplated on the four dimensions of nutritional sustainability, as well as one of the methodological proposals to assess the sustainability of typical agro-foods of MedDiet. Environmental sustainability was the most well-studied dimension, while no study focuses on the socio-cultural dimension of sustainability. Our study reviewed for the first time the assessment of nutritional sustainability of MedDiet. To the best of our knowledge, no research has been made assessing MedDiet in all the dimensions of the complex concept, that is nutritional sustainability. Integrating health and nutrition, environmental, economic, and socio-cultural considerations across scales and contexts can offer a more complete understanding of the opportunities and barriers to achieving nutritional sustainability not only in MedDiet but also in other dietary patterns and food products.

Keywords: Mediterranean diet (MedDiet), nutritional sustainability, health indicator, environmental footprint (EF), diet impact

INTRODUCTION

Recently, the EAT-Lancet Commission identified food as the single strongest lever to optimize human health and environmental sustainability on Earth (1). Sustainable diets have emerged as a key issue in nutrition and public health (2). The notion of "sustainable diets" was proposed in 1986 by Gussow and Clancy to endorse diets that would be healthier for the environment as well as for consumers (3). Abandoned for several years, the interest in this concept has been gaining attention recently. In 2010, FAO in collaboration with Bioversity International reached a scientific position on the definition of sustainable diets: "Sustainable diets are those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe, and healthy; while optimizing natural and human resources" (4).

Sustainable diets are person-centered and are the last event in a chain that encompasses production, processing, distribution, and consumption of food and in their turn, define a food system. A high level panel of experts of the Committee on World Food Security defined a sustainable food system as "a food system that ensures food security and nutrition for all in such a way that the economic, social, and environmental bases to generate food security and nutrition of future generations are not compromised" (5). Sustainable diets and sustainable food systems are two closely interrelated notions. The contribution of the diet to the sustainability of the food system is what characterizes the sustainability of the diet, and sustainable diets are not only an objective but an essential means to achieve a sustainable food system (6).

Nutritional sustainability is defined as "the ability of a food system to provide sufficient energy and the amounts of essential nutrients required to maintain good health of the population without compromising the ability of future generations to meet their nutritional needs" (7, 8) and combines in one concept, aspects from sustainable diets and sustainable food systems. Nutritional sustainability is an interesting concept that not only sets environmental sustainability as a baseline level for balanced nutrition but also aims for the sustainability of the food system and calls for a more accurate assessment of the capacity of the environment for the development of more efficient nutrition solutions balanced within the limits of sustainability (8). Similar to the concept of sustainable food systems, nutritional sustainability also recognizes that ecological, social, and economic aspects must be balanced to support the sustainability of the overall food system but also acknowledged its contribution to health and nutrition present in the definition of sustainable diets (4–7).

Mediterranean diet (MedDiet) is often used as an example of a sustainable diet (9, 10) that promotes a sustainable food system (11, 12). MedDiet is a dietary pattern rich in cereals, fruits, vegetables, legumes, tree nuts, seeds, and olives, with olive oil as the principal source of added fat, along with high to moderate intakes of fish and seafood, moderate consumption of eggs, poultry and dairy products (cheese and yogurt), low consumption of red meat, and a moderate intake of alcohol (mainly wine during meals) (13, 14). MedDiet is the heritage of millennia of exchanges of people, cultures, and foods of all countries around the Mediterranean basin. It has been the basis of food habits during the 20th century in all countries of the region, based on Mediterranean agricultural and rural models (13). According to UNESCO, MedDiet involves a set of skills, that concerns, not only the sharing and consumption of food, but also knowledge, rituals, and traditions concerning crops, harvesting, fishing, animal husbandry, conservation, processing, and cooking. MedDiet is a way of life guided by respect for diversity, which emphasizes values of hospitality, neighborliness, intercultural dialogue, and creativity (15). Since its identification, MedDiet has been considered a healthy diet. Robust evidence suggests that adherence to the MedDiet is associated with a reduced risk of overall mortality, cardiovascular diseases (CVDs), coronary heart disease, myocardial infarction, overall cancer incidence, neurodegenerative diseases, and diabetes (16).

Based on its intrinsic characteristics, MedDiet presents several sustainability benefits. Considering the three dimensions of sustainability (environmental, social, and economic), MedDiet presents low environmental impacts, is characterized by high sociocultural food values, and allows for positive local economic returns. Furthermore, when talking about dietary patterns, a fourth dimension has been added, which is health and nutritional sustainability, which MedDiet also fulfills with major health and nutrition benefits (17).

Assessing the sustainability of the diets and/or nutritional sustainability is a challenging task. Despite the increased attention paid to nutritional sustainability and/or sustainable diets and the importance of clearly and comprehensively measured sustainability, it is not clear how the different components of sustainable diets and/or nutritional sustainability are prioritized or operationalized (18). The assessment of MedDiet sustainability has not been different. To the best of our knowledge, previous studies have not systematically examined the methodological assessment of MedDiet nutritional sustainability. Previous reviews have emphasized that studies have examined exclusively the environmental impacts of diets rather than assessing the many other components of sustainable diets (19, 20). Cross-disciplinary studies on environmental, economic, socio-cultural, and health-nutrition sustainability dimensions of the Mediterranean diet are a critical need (10).

The overall aim of this study is to provide a summary of the methodological assessment of nutritional sustainability in the context of MedDiet available in the scientific literature. More specifically, the objectives are to

(i) analyze the methodological differences in the assessment of nutritional sustainability,
(ii) identify methods to combine nutrition indicators and sustainability indices, and to
(iii) explore the comprehensiveness of those indices to assess nutritional sustainability.

To our knowledge, this is the first scoping review focusing on the methodological assessment of MedDiet nutritional sustainability, filling a crucial gap in the literature that can inform the state of the art regarding the cross-disciplinary assessment of MedDiet nutritional sustainability.

METHODS

Literature Search

The study design and analysis of this scoping review follow the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (21). The review protocol was not registered.

The search was made in Scopus and PubMed in October 2021 using the following search queries:

- Scopus: ;(title-abs-key (food) or title-abs-key (diet*) or title-abs-key (nutri*) and title-abs-key (sustain*) and title-abs-key (Mediterranean)];
- PubMed: ((((food[title/abstract]) or (diet*[title/abstract])) or (nutri* [title/abstract])) and (sustain*[title/abstract])) and (Mediterranean [title/abstract]).

The search strategy was constructed based on the population, intervention, comparison, and outcome (PICO) framework. **Table 1** provides a description of the PICO framework.

No time frame was set during the search to obtain a more comprehensive search of relevant published literature data. The literature search was limited to journal articles. Title, abstract, and keywords were searched in Scopus and, title and abstract were searched in PubMed. Articles reviewed were limited to English-language articles published in peer-reviewed scientific journals. Study protocols, gray literature, and conference abstracts were excluded. Articles included in this review were further limited to those using the following methodology:

TABLE 1 | Population, intervention, comparison, and outcome (PICO)framework.

Population or problem	Adults and youth aged 2 years and older
Intervention or Exposure	MedDiet
Comparison	Other dietary pattern or lower adherence to MedDiet
Outcome	Sustainability • Environmental indicators • Economic indicators • Socio-cultural indicators • Health-nutrition indicators

(i) Assessment of MedDiet sustainability (alone or in comparison with other dietary patterns) using dietary consumption data;
(ii) Assessment of MedDiet sustainability (alone or in comparison with other dietary patterns) based on dietary scenarios;
(iii) Methodological proposals to assess the MedDiet nutritional sustainability of food, meals, or diets.

Determination of articles that met these inclusion criteria was made based on the information available in the titles and abstracts of the publications and in a later stage based on full text.

Synthesis of Results

The assessment of reviewed articles was made from a research approach and methodological perspective. Studies that assessed the sustainability of MedDiet based on food consumption surveys data (3.2), studies that explored the sustainability of MedDiet resorting to dietary scenarios based on recommendations (3.3–dietary scenarios studies), studies with a mixed approach (3.4–food consumption surveys and dietary scenarios) and proposals of methodological approaches to assess MedDiet nutritional sustainability (3.5) were summarized.

All relevant information from eligible studies was collected using a data extraction sheet. For the studies that assessed the sustainability of MedDiet based on dietary consumption data, the following data were extracted: (i) study design (cross-sectional, longitudinal, or experimental), (ii) participant demographics (type of participants, sample size, and location), (iii) dietary patterns analyzed, (iv) sustainability indicators, and (v) findings. For the studies that resorted to dietary scenarios and for studies with a mixed approach, the study design was not relevant; therefore, it was not reported. Also, for the studies that resorted to dietary scenarios, participant demographics were not applicable; nevertheless, the location of the study was recorded. Given the type of works to be included in this review, no critical appraisal was performed.

RESULTS

Study Selection and Characteristics

The literature search identified 1,528 articles after duplicates removal. A total of 148 articles were excluded based on title and abstract screening. Full texts of the remaining 48 articles were

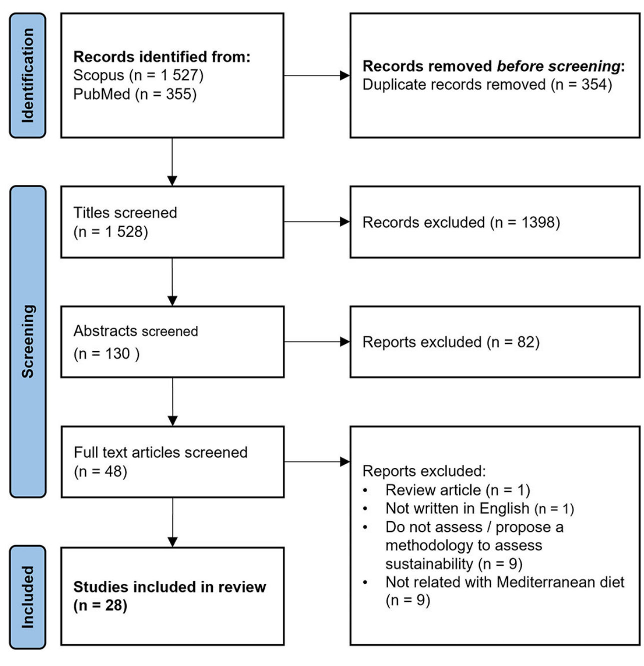

FIGURE 1 | Literature search and selection of articles in the review.

examined in detail accounting for the inclusion and exclusion criteria. From those, 28 studies met the inclusion criteria. Details are outlined in the PRISMA flow diagram of the selection process (**Figure 1**).

The identified studies were published between 2012 and 2021. A total of 28 records met the eligibility criteria: 9 studies assessed the sustainability of MedDiet based on food consumption data (22–30), 11 studies assessed the MedDiet sustainability using dietary patterns recommendations (dietary scenarios) (31–41), 4 studies used a mixed approach (dietary consumption data vs. dietary scenarios) (42–45), and 4 studies proposed methodological approaches to assess sustainability within the MedDiet (46–49). All 28 articles reviewed were found to be transparent and provided the information required for our analysis.

MedDiet Sustainability Based on Dietary Consumption Data

Out of the 28 articles included in this review, 9 analyzed MedDiet sustainability based on dietary consumption data. Relevant information from these articles is summarized in **Table 2**.

Most of the studies were from countries located in the Mediterranean basin; two studies were conducted in Italy (24, 25), two in Lebanon (27, 28), two in Spain (26, 29), one in France (30), one in Albania (22), and one was multicenter (the Netherlands, the United Kingdom, Germany, and Spain) (23). Five of the nine studies included in this analysis were cross-sectional observational studies (22, 25, 27, 28, 30), three were longitudinal observational studies (24, 26, 29), and one was experimental (clinical trial) (23). Most of the studies were conducted using the dietary consumption data from adults, and one study was conducted using the dietary consumption data from school children (24). Sample sizes vary from 289 to 22,866 subjects for the studies including adults and 172 subjects for the study including school children. The identified studies were conducted between 2017 and 2021.

Dietary Patterns

Two studies assessed the sustainability associated with the adherence to MedDiet (24, 27), one study assessed the sustainability of the adherence to MedDiet in combination with organic food consumption (30), one study assessed alterations in sustainability indicators resulting from an intervention promoting MedDiet (23), and the remaining studies compared MedDiet with other dietary patterns. The dietary patterns compared with MedDiet included the Dietary Approach to Stop Hypertension (DASH), the EAT-Lancet reference diet, the Nordic diet, the Western dietary pattern, the provegetarian dietary pattern, the high-protein dietary pattern, the dietary pattern based on the Alternate Healthy Eating Index (AHEI), and the Dietary Quality Index-International (DQI-I). The above-mentioned studies used dietary consumption data to calculate the adherence to each dietary pattern.

Sustainability Indicators

Most of the studies used life cycle assessments to obtain environmental sustainability indicators; therefore, reports of sustainability indicators related to the potential environmental impacts of food products were included in the dietary patterns during their entire life cycle (23–29). The environmental sustainability indicators included Greenhouse gases (GHGs) emissions (23, 25, 27–29), land use (23, 25, 29), energy use (23, 25, 27–29), water use (25, 27–29), carbon footprint (CF) (24), and ecological footprint (EF) (24). The CF is the amount of CO_2 equivalent emissions (expressed in g CO_2 eq) produced during the life cycle, and the EF is the area of land needed to regenerate the applied resources (expressed in m^2). Four studies included indices obtained from environmental sustainability indicators as outcomes (23, 25, 26, 29). Grasso et al. (23) used the pReCiPe score, which is a weighted combination of GHGs emissions, land use, and fossil energy use. A sustainability score was applied by Gosso et al. (25) and Fresán et al. (29), and it was calculated by assigning 0 or 1 points to water, land, and energy use and GHGs emissions of each food product, using the sex-specific medians as the cut-offs (0 for upper values and 1 for lower ones). The sustainability score resulted from the sum of each component ranged from a total of 0 to 4 points, with higher scores indicating a less environmental impact. Fresán et al. (26) used a similar index, called the environmental footprints index, calculated in the same way as the sustainability score but in which participants were classified into quartiles, each of them ranking from 1 to 4. Similarly, the total environmental footprints index was created by summing the quartile values of all the four indicators (land, water, energy use, and GHGs emission); therefore, environmental footprints index ranked from 4 to 16 points.

TABLE 2 | Summary of studies reporting MedDiet sustainability using dietary consumption data.

References	Study type	Participants; n	Location	Dietary patterns	Sustainability indicators	Findings
Llanaj et al. (22)	Cross-sectional observational study	Young adults; n = 289	Albania	• MedDiet • DASH • EAT-Lancet reference diet	• Cost	• Better adherence to DASH, EAT-Lancet reference diet or MedDiet was not associated with dietary cost.
Grasso et al. (23)	Experimental (Clinical trial)	Adults; *n* = 744	Netherlands, United Kingdom, Germany and Spain	• Food-related behavioral activation therapy applying MedDiet guidelines (*n* = 373) • No intervention (*n* = 371)	• GHGs emissions • Land use • Energy use • pReCiPe score	• The intervention group reported increased intakes of vegetables, fruit, fish, pulses/legumes and whole grains, and decreased intake of sweets/extras relative to control group. • This effect on food intake resulted in no change in GHGs emissions, land use, and pReCiPe score, but a relative increase in fossil energy use. • A shift toward a healthier Mediterranean-style diet does not necessarily result in a diet with reduced environmental impact in a real-life setting.
Rosi et al. (24)	Longitudinal observational study	School children; *n* = 172	Italy	• MedDiet	• CF • EF	• CF and EF were higher during winter, and the main dietary contributors were red and processed meat for both indexes. • A small positive correlation was observed between adherence to the MD and total CF and EF.
Grosso et al. (25)	Cross-sectional observational study	Adults; *n* = 1,806	Italy	• MedDiet • DASH • Nordic diet • AHEI • DQI-I	• Land use • Water use • Energy use • GHGs emissions • Sustainability score	• Animal products (dairy, egg, meat, and fish) represented more than half of the impact on GHG emissions and energy requirements. Meat products were the stronger contributors to GHG emissions and water use. Dairy products were the stronger contributors to energy use. Cereals were the stronger contributors to land use. • All patterns investigated, except for DASH, were linearly associated with the sustainability score. • Higher adherence to MedDiet and AHEI was associated with lower GHGs emissions. • DQI-I was associated with lower land use. • Nordic diet was associated with lower land and water use.
Fresán et al. (26)	Longitudinal observational study	University graduates; *n* = 18,429	Spain	• MedDiet • Western dietary pattern • Provegetarian dietary pattern	• Rate advancement period (healthiness) • Cost • Environmental footprints index • Overall sustainable diet index	• The MedDiet exhibited the best rate advancement period (3.10 years gained for the highest *vs.* the lowest quartile), while the Western pattern was the unhealthiest pattern (1.33 years lost when comparing extreme quartiles). • Regarding EF index, Provegetarian pattern scored best when comparing extreme quartiles, whereas the Western pattern was the most detrimental pattern. • Regarding monetary costs, the Western pattern was the most affordable pattern (€5.87/day, for the upper quartile), while the MedDiet was the most expensive pattern (€7.52/day). • The MedDiet was the most overall sustainable option, closely followed by the Provegetarian pattern.
Naja et al. (27)	Cross-sectional observational study	Adults; *n* = 2,610	Lebanon	• MedDiet	• Water use • Energy use • GHGs emissions	• Two of the four MedDiet scores were associated with lower water use. • For GHGs emissions, significant inverse associations were observed with all MedDiet scores. • Energy use was not associated with MedDiet scores.

(Continued)

TABLE 2 | Continued

References	Study type	Participants; n	Location	Dietary patterns	Sustainability indicators	Findings
Naja et al. (28)	Cross-sectional observational study	Adults; $n = 337$	Lebanon	• Lebanese-MedDiet pattern • Western dietary pattern • High-Protein dietary pattern	• Water use • Energy use • GHGs emissions.	• The Lebanese-MedDiet had the lowest water use and GHGs emissions per 1,000 Kcal. • The highest energy use was that of the Western dietary pattern, followed by the Lebanese-MedDiet and the High-Protein dietary pattern.
Fresán et al. (29)	Longitudinal observational study	University graduates; $n = 20,363$	Spain	• MedDiet	• Land use • Water use • Energy use • GHGs emission • Sustainability score	• Better adherence to the MedDiet was associated with lower land use, water consumption, energy consumption and GHGs emission.
Seconda et al. (30)	Cross-sectional observational study	Adults; $n = 22,866$	France	• Conventional consumers and non-MedDiet followers (Conv–NoMedDiet; $n = 14,266$) • Conventional consumers and MedDiet followers (Conv–MedDiet; n = 3,498) • Organic consumers and non-MedDiet followers (Org–NoMedDiet; $n = 2,532$) • Organic consumers and MedDiet followers (Org–MedDiet; $n = 2,570$)	• PANDiet • mPNNS-GS • Dietary diversity score • Plant/animal protein intake ratio • Cost	• The adherence to nutritional recommendations was higher among the Org–MedDiet and Conv–MedDiet groups compared to the Conv–NoMedDiet group (using the mPNNS-GS). • The mean plant/animal protein intake ratio was 1.38 for the Org–MedDiet group versus 0.44 for the Conv–NoMedDiet group. • The average cost of the diet of Org–MedDiet participants was the highest. • The importance of promoting the MedDiet combined with organic food consumption is highlighted for individual health and environmental aspects but challenges regarding the cost remain.

MedDiet, Mediterranean diet; DASH, Dietary Approach to Stop Hypertension; AHEI, Alternate Healthy Eating Index; DQI-I, Diet Quality Index International; Conv–NoMedDiet, Conventional consumers and non-MedDiet followers; Conv–MedDiet, Conventional consumers and MedDiet followers; Org–NoMedDiet, Organic consumers and non-MedDiet followers; Org–MedDiet, Organic consumers and MedDiet followers; GHGs, Greenhouse Gases; CF, Carbon Footprint; EF, Ecological Footprint; PANDiet, Probability of Adequate Nutrient intake; mPNNS-GS, modified Programme National Nutrition Santé-Guidelines Score.

Three studies included the cost of diet as an economical sustainability indicator (22, 26, 30). All the studies used the daily cost of diet as the main indicator; however, Seconda et al. (30) also reported the share of the budget allocated to foods by dividing the total cost of diet by the income reported by the participants.

Health-nutrition sustainability indicators were presented in two studies (26, 30). The rate of advancement period was used by Fresán et al. (26) as a heath indicator that measures the time by which a rate of a specific outcome is advanced or it is postponed among exposed subjects compared to unexposed individuals, conditional on being free from the outcome at the baseline. Nutrition indicators were used by Seconda et al. (30) to assess diet quality. Briefly, plant/animal protein ratio and three *a priori* dietary scores were computed: a diet quality index based on the Probability of Adequate Nutrient (PANDiet) intake that reflects the adequacy between nutrient intakes and French recommendations for 24 nutrients, the modified Programme National Nutrition Santé-Guidelines Score (mPNNS-GS) that reflects the level of adherence to the French food-based recommendation defined by the Programme National Nutrition Santé, and the dietary diversity score that evaluates the number of food groups consumed per day.

One index gathered the impact of the daily diet on health, environmental footprints index, and monetary costs; the overall sustainable diet index was designed and reported by Fresán et al. (26). Briefly, for the three aspects, a score from 0 to 3 points was given for each of them, the less suitable value for health, environment, and economy was given 0 points; 3 points for the healthiest daily diet, the one that produced less environmental footprints and the cheapest one. Proportional scores were given for the rest of the values. Summing those three values, the overall sustainable diet index was obtained ranging from 0 to 9 points, with 0 being the less suitable diet and 9 being the most appropriate diet.

Main Findings

The most consistent finding of the studies exploring sustainability based on dietary consumption data indicates that adherence to MedDiet is associated with higher environmental sustainability.

Naja et al. (27), Rosi et al. (24), and Fresán et al. (29) explored the association of the adherence to MedDiet with environmental sustainability indicators. In a sample of 2,610 adults from Lebanon, Naja et al. (27) found that higher adherence to MedDiet was associated with lower water use, lower GHGs emissions, and it was not associated with energy use. Fresán et al. (29) reported that higher adherence to MedDiet was associated with lower use of land, water, and energy, and reduced GHGs emissions. Surprisingly, Rosi et al. (24) found that higher adherence to MedDiet in a sample of 172 Italian school children was positively associated with CF and EF.

Grasso et al. (23) investigated whether food-related behavioral activation therapy applying MedDiet guidelines altered the food intake and the environmental impact of the diet in overweight adults with subsyndromal symptoms of depression. The intervention group altered food intake toward MedDiet; however, this effect resulted in no change in GHGs emissions, land use, and pReCiPe score, and a relative increase in the use of fossil energy.

Grosso et al. (25) studied the environmental impact of dietary patterns in an Italian cohort. The authors found that, except for DASH, the adherence to healthy dietary patterns (MedDiet and Nordic diet) and higher diet quality indices (AHEI and DQI-I) were associated with higher sustainability scores. They also found that higher adherence to MedDiet and AHEI was associated with lower GHGs emissions. Naja et al. (28) also found that adherence to MedDiet was associated with lower water use and GHGs emissions per 1,000 Kcal when compared to Western and high-protein dietary patterns. The environmental impact of the Western dietary pattern was also compared with MedDiet in a study from Fresán et al. (26), and it was shown that the Western dietary pattern was the most detrimental one for the environment, while the Provegetarian dietary pattern was the most beneficial one followed by the MedDiet.

Several of the studies also presented data on the contribution of food/food groups to the environmental sustainability indicators. Rosi et al. (24) showed that animal-based products represented 50% or more of the impact on the CF and EF. Similar results were observed by Grosso et al. (25) in which animal products represented more than half of the impact on GHG emissions, water use, and energy requirements. Naja et al. (28) reported that, within the MedDiet, whole dairy products had the highest percentage of contribution to water use, while vegetables contributed most to energy use and GHGs emissions. The authors explained these results by the relatively high consumption of vegetables within the Lebanese MedDiet and the fact that the production of vegetables requires more energy use and GHGs emissions than grains and fruits. In a later study (27), it was reported that red meat was the greatest contributor to water use, sugar-sweetened beverages were the main contributors to energy use, and red meat was the food group with the highest contributions to GHGs emissions.

Economic sustainability was assessed through the monetary cost. Llanaj et al. (22) analyzed the cost of the adherence to recommended dietary patterns and found that higher adherence to DASH, EAT-Lancet reference diet, or MedDiet was not associated with significant differences in cost. Fresán et al. (26), showed that MedDiet was the most expensive diet compared to the Western and Provegetarian dietary patterns. Seconda et al. (30) explored the cost of the adherence to MedDiet in combination with the consumption of organic food and observed that the average cost of consuming a MedDiet combined with organic food was the highest (MedDiet without organic food or no MedDiet compliance with or without organic food).

The health-nutrition pillar of sustainability was assessed by the study by Fresán et al. (26) and Seconda et al. (30). Fresán et al. (26) showed that the highest quartile of adherence to MedDiet exhibited the best rate advancement period (3.10 years gained), while the highest quartile of adherence to the Western dietary pattern showed the worst rate advancement period (1.33 years lost). Seconda et al. (30) demonstrated that the highest adherence to MedDiet (with or without combination with organic food) was associated with higher diet quality, adherence to recommendations, dietary diversity, and higher plant/animal protein ratio.

Fresán et al. (26) used an index that gathered the impact of all the analyzed aspects (health, environmental footprints, and monetary costs), the overall sustainable diet index. Using the overall sustainable diet index, the authors showed that MedDiet was the most sustainable option in comparison with Western and Provegetarian dietary patterns.

MedDiet Sustainability Based on Dietary Scenarios

Out of the 28 articles included in this review, 11 analyzed MedDiet sustainability based on the models of dietary patterns or recommendations (dietary scenarios) (31–41). Relevant information from these articles is summarized in **Table 3**.

Studies were conducted using the recommendations or dietary patterns from countries located in the Mediterranean basin, Netherlands, and the United States; briefly, one study was conducted in the Netherlands (37), three studies in the United States (34, 35, 39), seven studies in the Mediterranean basin (32, 33, 36, 38–41), and one study with no specific location discernible (31). The identified studies were published between 2012 and 2021.

Dietary Patterns

Most of the studies compared the MedDiet scenario with other dietary patterns or recommendations, such as, the European dietary pattern (31), the Western dietary pattern (31), EAT-Lancet reference diet (32), the Southern European Atlantic Diet (SEAD) (33), the Spanish Dietary Guidelines (NAOS) (33), Healthy US diet (34, 35), Lacto-ovo vegetarian diet (34), typical American diet (34, 39), healthy vegetarian dietary pattern (35), New Nordic diet (36, 37), optimized Low Lands diet (37), Italian average diet (40), healthy consumption pattern (40), vegetarian consumption pattern (40), status-quo diet (Iran) (41), WHO recommended diet (41), and the diet recommended by World Cancer Research Fund (WCRF) (41). One study explored the sustainability of different MedDiet scenarios, such as Healthy MedDiet, healthy pesco-vegetarian MedDiet, and healthy vegetarian MedDiet (38).

Sustainability Indicators

Most of the studies reported environmental sustainability indicators, including land use (31, 37), water use (31, 35), GHGs emissions (31, 36, 37), eutrophication potential (31), water footprint (WF) (32, 33, 39), CF (33, 40), global warming potential (34, 35), freshwater eutrophication (35), marine eutrophication (35), particulate matter or respiratory organics (35), and energy use (40). The WF is an indicator of freshwater consumption (from rainfall, surface, and groundwater) that looks at direct and indirect water use of a producer or consumer and water resources appropriation (expressed in liters) (33). One study used a combined GHGs emissions-land use (GHGE-LU) score that was defined as the average of the GHGs emissions and LU score per diet (37, 43). One study reported the variation in environmental load (emission of GHGs, such as CO_2, CH_4, and N_2O) expected in case of change for different dietary scenarios (41).

Sustainability was also assessed in the dimensions of economy and health nutrition. Economic sustainability was assessed in three studies, using the daily cost of diet (expressed in €·person^{-1} · day^{-1} or €·family^{-1} · month^{-1}) (33, 40) or total changes in output (41). One study assessed the nutritional quality through the Nutrient Rich Foods Index 9.3 (NRF9.3) and Nutrient Quality Index (NQI), and satiety was assessed by the FullnessFactor^{TM} (FF) (34). van Dooren et al. (37) used a health score to assess the healthiness of diets, based on the adequacy of the Dutch recommendations of ten nutritional indicators (food, nutrients, or energy).

Main Findings

Studies using dietary scenarios consistently found MedDiet as a sustainable pattern; although, it was not always considered superior to other healthy dietary patterns.

Vanham et al. (32) estimated the WF of MedDiet and EAT-Lancet reference diet in nine Mediterranean countries. The authors reported that the EAT-Lancet reference diet consistently reduces the current WF of the analyzed countries while MedDiet reduces WF to a smaller extent or even increases it. In a previous study, Vanham et al. (38) compared the WF of MedDiet scenarios with the reference situation in 13 Mediterranean cities and demonstrated that the adoption of MedDiet patterns (either including meat, pesco-vegetarian, or vegetarian) would reduce WF. Blas et al. (39) also compared the WF of MedDiet with the American diet and reported that the American diet has a 29% higher WF. The authors also reported that a shift to the Mediterranean diet would decrease the WF in the US, while a shift toward an American diet in Spain will increase the WF. Despite presenting a lower WF when compared to a typical American diet, the MedDiet presented a higher water depletion, and higher freshwater and marine eutrophication when compared with the Healthy US-style dietary pattern and the healthy vegetarian dietary pattern according to the study by Blackstone et al. (35). In this study (35), MedDiet presented a slightly lower global warming potential and land use, and slightly higher particulate matter than the Healthy US-style dietary pattern; however, MedDiet presented the worst environmental performance in all indicators when compared to healthy vegetarian dietary pattern. The authors mentioned that reliance on plant-based protein and eggs in the healthy vegetarian dietary pattern vs. emphasis on animal-based protein in the other patterns was a key driver of differences. A lacto-ovo vegetarian diet also performed better than other dietary patterns analyzed in the United States, including the MedDiet.

Chapa et al. (34) showed that Lacto-ovo vegetarian diet generated the lowest global warming potential regardless of the nutritional quality and satiety. Considering the nutritional quality and satiety, the authors concluded that high satiety foods can help prevent overconsumption and thus improve dietary CF. The authors also identified animal products, including meat and dairy, and discretionary foods as the

TABLE 3 | Summary of studies reporting MedDiet sustainability using dietary scenarios.

References	Location	Dietary scenarios	Sustainability indicators	Main findings
Belgacem *et al.* (31)	Not applicable	• MedDiet • European dietary pattern • Western dietary pattern	• Land use • Water use • GHGs emissions • Eutrophication potential	• A shift from the European to the Mediterranean dietary pattern would lead to 10 m^2/capita/day land savings, 240 L/capita/day water savings, 3 kg CO_2/capita/day reduction in greenhouse gas emissions, and 20 g PO_4eq/capita/day reductions in eutrophication potential. • A shift from the Western to the Mediterranean dietary pattern would lead to 18 m^2/capita/day land savings, 100 L/capita/day water savings, 4 kg CO_2/capita/day reduction in greenhouse gas emissions, and 16 g PO_4eq/capita/day reduction in eutrophication potential.
Vanham *et al.* (32)	Nine Mediterranean countries (Spain, France, Italy, Greece, Turkey, Egypt, Tunisia, Algeria and Morocco)	• MedDiet • EAT-Lancet reference diet	• WF	• The EAT-Lancet diet requires less water resources than the MedDiet. In terms of water resources use, adherence to the former is thus more beneficial than adherence to the latter. • The EAT-Lancet diet reduces the current WF for all nations consistently, within the range−17–48%, whereas the MedDiet reduces the WF of the European countries, Turkey, Egypt and Morocco within the range of−4-−35%. • For the Maghreb countries Tunisia and Algeria, the Mediterranean diet WF is slightly higher compared to the current WF.
Gonzalez-García *et al.* (33)	Spain	• MedDiet • SEAD • NAOS	• CF • WF • Cost	• The dietary energy recommendation of the SEAD is greater than that of MedDiet and NAOS (11 and 15%, respectively), and SEAD also has greater animal source food content than the other two diets. • SEAD has a concomitantly higher CF, WF and cost scores in comparison with MD (+30, +23, and +21%, respectively) and NAOS (+15, +9, and +21%, respectively). • Adjusting recommendations to meet the suggested Spanish adult dietary energy of 2,228 kcal·capita^{-1} · day^{-1} changed the environmental profiles of the diets, and the NAOS has the highest environmental impact. • Isocaloric diets had approximately the same cost. • Regardless of the dietary scenario, better scores were identified for the Spanish recommendations analyzed than those reported for other healthy diets identified in Europe.
Chapa *et al.* (34)	United States	• MedDiet • Healthy U.S. diet • Lacto-ovo vegetarian diet • Typical American diet	• NRF9.3 • NQI • FF • Global warming potential	• Vegetarian diets on average generated the lowest carbon footprint regardless of the NRF9.3, NQI and FF. • Animal products, including meat and dairy especially, and discretionary foods were identified as the specific food categories that contributed the most to the global warming potential.
Blackstone *et al.* (35)	United States	• MedDiet • Healthy US-style diet • Healthy vegetarian dietary pattern	• Global warming potential • Land use • Water use • Freshwater eutrophication • Marine eutrophication • Particulate matter or respiratory organics.	• The Healthy US-style dietary pattern and MedDiet pattern had similar impacts, except for freshwater eutrophication. • Freshwater eutrophication was 31% lower in the US pattern than the MedDiet pattern, primarily due to increased seafood in the MedDiet pattern. • All three patterns had similar water depletion impacts, with fruits and vegetables as major contributors. • For five of the six impacts, the Healthy vegetarian dietary pattern had 42–84% lower burdens than both the Healthy US-style dietary pattern and MedDiet pattern. • Reliance on plant-based protein and eggs in the Healthy vegetarian dietary pattern vs. emphasis on animal-based protein in the other patterns was a key driver of differences.

(Continued)

TABLE 3 | Continued

References	Location	Dietary scenarios	Sustainability indicators	Main findings
Ulaszewska *et al.* (36)	Italy	• MedDiet • New Nordic Diet	• GHGs emissions	• Consumption of high protein foods has a similar and comparable environmental impact to fruit and vegetable consumption. • Mediterranean Diet and New Nordic Diet had similar total values of GHG emissions.
van Dooren *et al.* (37)	Netherlands	• MedDiet • New Nordic Diet • Optimized Low Lands Diet	• GHGs emissions • Land use • Combined GHGE–LU Score • Health score	• An optimized Low Lands Diet has the same healthy nutritional characteristics (Health Score 123) as the Mediterranean Diet (122) and results in a lower environmental impact than the Mediterranean and New Nordic Diet (higher Combined GHGE-LU Score 121 vs. 90 and 91). • For optimized Low Lands Diet, GHGs emissions are 2.60 kg CO_2eq per day and land use are 2.86 m^2*year per day, which are the best scores of all diets analyzed.
• Vanham *et al.* (38)	• 13 Mediterranean cities (Dubrovnik, Lyon, Athens, Jerusalem, Genova, Pisa, Bologna, Reggio Emilia, Ljubljana, Manresa, Zaragoza, Ankara and Istanbul)	• Healthy MedDiet • Healthy pesco-vegetarian MedDiet • Healthy vegetarian MedDiet	• WF	• Compared to reference situation, adoption of Healthy MedDiet (including meat), leads to WF reductions of −19–43%. The Healthy pesco-vegetarian MedDiet leads to WF reductions of −28–52%. The Healthy vegetarian MedDiet leads to WF reductions of −30–53%.
Blas et al. (39)	Spain and United States	• MedDiet • Typical American diet	• WF	• American diet has a 29% higher WF in comparison with the MedDiet, regardless of product's origin. • A shift to a Mediterranean diet would decrease the WF by 1,629 L/person/day in the US. A shift toward an American diet in Spain will increase the WF by 1,504 L/person/day.
Pairotti *et al.* (40)	Italy	• MedDiet • Italian average diet • Healthy consumption pattern • Vegetarian consumption pattern	• Cost • Energy use • CF	• When compared with the Italian average diet, the MedDiet revealed an improvement in environmental performance of 95.75 MJ (2.44%) and 27.46 kg CO_2 equivalent (6.81%) per family. • The best overall environmental performance can be found with the vegetarian diet in which energy consumption is 3.14% lower and the carbon footprint 12.7% lower than the Italian average diet.
Rahmani *et al.* (41)	Iran	• Status-quo diet • MedDiet • WHO recommendations • WCRF recommendation	• Total changes in output • Environmental load	• Compared to Sattus-quo diet, total changes in output in WHO, WCRF and Mediterranean dietary scenarios were calculated to be 7010.1, 4802.8 and 3330.8 billion Rials respectively. • The environmental load increased for three dietary scenarios in comparison with the status-quo diet. The greatest and smallest environmental load occurred in WHO and Mediterranean dietary scenarios respectively.

MedDiet, Mediterranean diet; SEAD, Southern European Atlantic diet; NAOS, Spanish dietary guidelines; WHO, World Health Organization; WCRF, World Cancer Research Fund; GHGs, Greenhouse Gases; WF, Water Footprint; CF, Carbon Footprint; NRF9.3, Nutrient Rich Foods Index 9.3; NQI, Nutritional Quality Index; FF, Fullness Factor™; GHGE-LU, Greenhouse Gases Emissions-Land Use.

specific food categories that contributed the most to the global warming potential. Similarly, Pairotti et al. (40) found that, when compared with the Italian average diet, the MedDiet revealed an improvement in energy use and in CF. Despite that, compared to the Italian average diet, the best overall environmental performance was found with the vegetarian diet in which energy use was 3.14% lower and the CF was 12.7% lower.

Gonzalez-García et al. (33) found that MedDiet had a lower CF and WF than SEAD and NAOS, the two recommended healthy dietary patterns in Spain. The SEAD presented the higher CF and WF explained by the greater animal source food content present in that dietary pattern. Belgacem et. al (31) compared three dietary scenarios and found that a shift from the European or Western dietary pattern to the MedDiet would lead to land and water savings, reduction in GHGs emissions, and eutrophication potential. Ulaszewska et al. (36) found comparable values of GHGs emissions in the MedDiet and the New Nordic diet. On the other hand, Rahmani et al. (41) observed that, in Iran, changing from the status-quo diet to MedDiet would increase the environmental load. Van Dooren et. al (37) noticed that an optimized low lands diet would result in a lower environmental impact (lower GHGs emissions, lower land use, and higher combined GHGE-LU score) with similar nutritional characteristics (measured by the health score) as the MedDiet.

Gonzalez-García et al. (33) analyzed the economic sustainability and, considering the isocaloric diets, the MedDiet, SEAD, and NAOS presented approximately the same cost. Pairotti et al. (40) indicated that MedDiet presented approximately the same cost as that of the Italian average diet.

Mixed Studies

Out of the 28 articles included in this review, 4 analyzed MedDiet sustainability based on the models of dietary patterns or recommendations (dietary scenarios) in comparison with the national food consumption surveys (42–45). Relevant information from these articles is summarized in **Table 4**.

Studies were conducted in countries located in the Mediterranean Basin and north of Europe; briefly, two studies were conducted in Spain, one study in Italy, and one study in the Netherlands. The identified studies were conducted between 2013 and 2019.

Dietary Patterns

All the studies compared MedDiet and other dietary patterns or recommendations with dietary consumption data obtained from national representative surveys. Apart from MedDiet, dietary scenarios explored in these studies included the Official "recommended" Dutch diet (43), the semi-vegetarian diet (43), the vegetarian diet (43), the vegan diet (43), and the Western dietary pattern (45). The dietary consumption patterns, obtained from the national representative samples, correspond to the Spanish dietary pattern (42, 45), the Dutch diet (43), and the real consumption of the Italian population (44).

Sustainability Indicators

Environmental sustainability indicators included WF (42, 44), GHGs emissions (43, 45), land use (43, 45), CF (44), EF (44), and WF (42, 44). One study used a combined GHGE-LU score (43).

Two studies included a health-nutrition indicator, the health score (43), and the multidimensional nutritional analysis (42). One study used an index that combines water use and nutritional values, the nutritional-water productivity (42). One study included the monetary cost (44).

Main Findings

MedDiet was consistently found to be a more sustainable option when a mixed approach, using dietary scenarios and data from food consumption surveys, was used.

Blas et al. (42) compared the WF of the Spanish dietary consumption with the MedDiet and demonstrated that a shift toward MedDiet would significantly reduce the WF. Furthermore, MedDiet presents better nutritional-water productivity than Spanish dietary consumption. The environmental sustainability of the Spanish dietary consumption was also compared with the sustainability of the adoption of a MedDiet pattern and a Western dietary pattern. Sáez-Almendros et al. (45) reported that increasing the adherence to the MedDiet pattern in Spain would reduce GHGs emissions, land use, energy consumption, and water consumption while increasing the adherence to a Western dietary pattern would increase all the descriptors.

van Dooren et al. (43) studied the environmental and health-nutrition sustainability of the Dutch diet and the other five dietary scenarios. Vegetarian diet and the vegan diet were the options with higher sustainability scores closely followed by MedDiet, which was the dietary pattern with the higher health score. MedDiet was considered, by the authors, the health focus option with a high GHGE-LU score.

When comparing the sustainability of the dietary consumption obtained through the Italian National Food Consumption Survey INRAN-SCAI 2005–06 with MedDiet recommendations, Germani et al. (44) showed that adherence to MedDiet may produce a lower environmental impact than the dietary consumption pattern of the Italian population. Despite the lower environmental impact, it was also shown that adherence to the MedDiet recommendations would result in a slightly higher cost when compared to the expenditure allocated to food by the Italian population, which may dampen the economic sustainability of MedDiet.

Proposals of Methodological Approaches to Assess MedDiet Nutritional Sustainability

Out of the 28 studies identified through our strategy, four were proposals of methodological approaches to assess the MedDiet nutritional sustainability. Two studies were proposals of methodological approaches to assess the nutritional sustainability of the MedDiet (46, 47), and two studies were methodological proposals to assess the nutritional sustainability of MedDiet typical agro-food (48, 49). The identified proposals were

TABLE 4 | Summary of studies reporting MedDiet scenario sustainability vs. other scenarios or dietary consumption.

References	Participants; *n*	Location	Dietary patterns	Sustainability indicators	Findings
Blas et al. (42)	National representative sample; *n* = 8,000 households	Spain	• MedDiet • Spanish dietary pattern	• Multidimensional nutritional analysis • WF • Nutritional-Water productivity	• Spanish dietary pattern has 3 times more meat-dairy-sweet and 1/3 fewer fruits-vegetables than MedDiet. • Due to the high embedded water content in animal products, a shift toward a MedDiet would reduce the consumptive WF about 750 l/capita day. • MedDiet has better water-nutritional efficiency (NWP) than the current one: it provides more energy, fiber, and nutrients per liter of consumptive water.
van Dooren et al. (43)	National representative sample; (1–97 years); *n* = 5,958	Netherlands	• MedDiet • Dutch diet • Official "recommender" Dutch diet • Semi-vegetarian diet • Vegetarian diet • Vegan diet	• Health score • GHGs emissions • Land use • Combined GHGE–LU Score	• Consumption of meat, dairy products, extras, such as snacks, sweets, pastries, and beverages, are largely responsible for low Combined GHGE–LU Score and simultaneously, these food groups contribute to low health scores. • The Mediterranean diet is generally the health focus option with a high Combined GHGE–LU Score. • Health and Combined GHGE–LU Score of all six diets go largely hand in hand.
Germani et al. (44)	National representative sample; (0.1-97.7 years); *n* = 3,323	Italy	• MedDiet • INRAN-SCAI consumption	• CF • EF • WF • Cost	• MedDiet produce a lower environmental impact than the food consumption of the Italian population (CF, EF and WF). • The monthly expenditure of the MedDiet is slightly higher in the overall budget compared to the expenditure allocated to food by the Italian population.
Sáez-Almendros et al. (45)	National representative sample; *n* = 6,000 households	Spain	• MedDiet • Spanish dietary pattern • Western dietary pattern	• GHGs emissions • Land use • Energy use • Water use	• Increasing adherence to the MedDiet pattern in Spain will reduce GHGs emissions (72%), land use (58%) and energy consumption (52%), and to a lower extent water consumption (33%). • Adherence to a western dietary pattern implies an increase in all the descriptors between 12 and 72%.

MedDiet, Mediterranean diet; INRAN-SCAI, Italian National Food Consumption Survey; WF, Water Footprint; NWP, Nutritional Water Productivity; GHGs, Greenhouse Gases; GHGE-LU, Greenhouse Gases Emissions-Land Use; CF, Carbon Footprint; EF, Ecological Footprint.

TABLE 5 | Summary of proposed methodological approaches to assess MedDiet sustainability.

References	Sustainability indicators	
Donini et al. (46)	**Biochemical characteristics of food** • Vegetable/animal protein consumption ratios • Average dietary energy adequacy • Dietary Energy Density Score • Nutrient density of diet **Food Quality** • Fruit and vegetable consumption/intakes • Dietary Diversity Score	**Environment** • Food biodiversity composition and consumption • Rate of Local/regional foods and seasonality • Rate of eco-friendly food production and/or consumption **Lifestyle** • Physical activity/physical inactivity prevalence • Adherence to the Mediterranean dietary pattern **Clinical Aspects** • Diet-related morbidity/mortality statistics • Nutritional Anthropometry.
Dernini et al. (47)	**Nutrition and health** • Diet-related morbidity/mortality • Fruit and vegetable consumption/intake • Vegetable/animal protein consumption ratio • Dietary energy supply/intakes • Dietary diversity score • Dietary energy density score • Nutrient density/quality score • Food biodiversity composition and consumption • Nutritional anthropometry • Physical activity prevalence **Environment** • Water footprint • Carbon footprint • Nitrogen footprint • Biodiversity	**Economy** • Food consumer price index: cereals, fruit, vegetables, fish and meat • Cost of living index related to food expenditures: cereals, fruit, vegetables, fish and meat • Distribution of household expenditure per groups: food • Food self-sufficiency: cereals, fruit and vegetables • Intermediate consumption in the agricultural sector: nitrogen fertilizers • Food losses and waste **Society and culture** • Proportion of meals consumed outside home • Proportion of already prepared meals • Consumption of traditional products (e.g., proportion of product under PDO (Protected Designation of Origin) or similar recognized traditional foods) • Proportion of mass media initiatives dedicated to the knowledge of food background cultural value

published between 2013 and 2018. Relevant information is summarized in **Tables 5**, **6**.

Sustainability of Dietary Patterns

Dernini et al. (47) proposed a methodological approach to assess the sustainability of dietary patterns using MedDiet as a case study. The methodological approach was based on the results of the participatory process, conducted in 2011 and 2012 by the International Centre for Advanced Mediterranean Agronomic Studies-Mediterranean Agronomic Institute of Bari (CIHEAM MAI-Bari) and FAO in collaboration with the National Agency for New Technologies, Energy and Sustainable Economic Development, Italy (ENEA), Italian National Research Council (CNR), the National Institute for Research on Food and Nutrition, Italy (INRAN), the International Interuniversity Study Centre on Mediterranean Food Cultures (CIISCAM), Bioversity International, and World Wildlife Fund for Nature, Italy (WWF-Italy), in which the three dimensions of sustainability (economic, social, and environmental) were added to nutrition and health. Within these, four thematic areas were identified as sets of sustainability indicators. The list of sustainability indicators for each criterion that was established is reviewed in **Table 5**.

The sustainability indicators on the nutrition and health thematic area included diet-related morbidity/mortality, fruit and vegetable consumption/intake, vegetable/animal protein consumption ratio, dietary energy supply/intakes, dietary diversity score, dietary energy density score, nutrient density/quality score, food biodiversity composition and consumption, nutritional anthropometry, and physical activity prevalence. On the environment thematic area, the sustainability indicators aggregated WF, CF, nitrogen footprint, and biodiversity. The set of sustainability indicators on the economy thematic area were food consumer price index, cost of living index related to food expenditures, distribution of household expenditure per food group, food self-sufficiency, intermediate consumption in the agricultural sector (nitrogen fertilizers), and food losses and waste. Identified indicators in the thematic area of society and culture were the proportion of meals consumed outside the home, the proportion of already prepared meals, consumption of traditional products (e.g., the proportion of products under the protected designation of origin or similar recognized traditional foods), and proportion of mass media initiatives dedicated to the knowledge of food background cultural value.

Later, in 2016, Donini et al. (46), in the sequence of the above-mentioned work, identified, refined, and summarized some of the most relevant nutritional indicators to measure the sustainability of food consumption and dietary patterns using the MedDiet as a case of study. Five main thematic areas were identified and included biochemical characteristics of food, food quality, environment, lifestyle, and clinical aspects. Among those areas, 13 nutrition indicators of sustainability were identified and the definition, the methodology, the background, data sources, limitations, and references for each indicator were provided.

Sustainability indicators proposed for the "biochemical characteristics of food" thematic area were vegetable/animal protein consumption ratios, average dietary energy adequacy, dietary energy density score, and nutrient density of the diet. For the "food quality" thematic area, the indicators were fruit and vegetable consumption/intakes, and dietary

TABLE 6 | Summary of proposed methodological approaches to assess the sustainability of MedDiet's typical agro-food products.

References	Sustainability indicators	

Azzini et al. (48)	**Business distinctiveness of agro-food companies and food safety**
	• Distinctiveness for agro-food companies
	  ◦ Application of EU regulations, specific national laws, and voluntary requirements.
	• Primary production, marketing, and labeling
	  ◦ Nutritional macro and micronutrient content regulated by national and EU laws.
		Foodstuffs: the healthy-nutritional sustainability
		• Nutritional sustainability index
		  ◦ Food specific nutritional indicators and their effects on health (Critical nutrients/"bioactive compounds," whose concentrations are considered for calculating the macro-indicator on the nutritional quality for each group of foods. For details see original publication)

Capone et al. (49)	**Environmental criterion / indicators**
	• Land use and management
	  ◦ Application of soil conservation practices
	  ◦ Soil erosion protection
	• Input of nitrogen fertilizers
	  ◦ Input of plant protection products
	  ◦ Use of agricultural machinery
	• Biodiversity
	  ◦ Crop diversity
	  ◦ Number of farm animal species
	  ◦ Tree plant density
	  ◦ Herbaceous plant diversity
	  ◦ Presence of cover crops
	  ◦ Legume crop density
	  ◦ Patch average area
	  ◦ Semi-natural habitat surface
	  ◦ Duration of rotation
	  ◦ Diversity of varieties and animal breeds
	• Varietal diversity
	  ◦ Number of plant varieties threatened by genetic erosion
	  ◦ Number of animal races (varieties)
	  ◦ Number of animal races (varieties) threatened by genetic erosion
	• Energy
	  ◦ Energy intensity
	• Climate change
	  ◦ Final Energy consumption
	  ◦ Mineral fertilizers consumption
	  ◦ Pesticide consumption
	• Lubricant consumption
	  ◦ Plastic material consumption
	  ◦ Use of off-farm animal feeds
	• Use of chemical inputs
	  ◦ Nitrogen consumption
	  ◦ Use of total phosphorus pentoxide
	  ◦ Use of fungicides
	  ◦ Use of insecticides and acaricides
	  ◦ Use of herbicides
	• Environmentally sound management of production scraps, by-products, and waste
	  ◦ Method for management of production scraps, by-products, and waste
	Economic criterion / indicators
	• Income level and stability
	  ◦ Number of products and services produced by the farm
	  ◦ Distribution of the turnover among different products and services
	  ◦ Heterogeneity or affinity of products and services supplied
	  ◦ Index of commercial riskiness–suppliers
	  ◦ Index of commercial riskiness–customers
		Economic criterion / indicators (continued)
		• Labor and employment
		  ◦ Index of localization
		• Investment
		  ◦ Specific investment for the improvement of sustainability performance
		• Profitability and productivity of production factors
		  ◦ Index of gross profitability per labor unit
		  ◦ Rate of return on invested capital
		  ◦ Enhancement rate
		  ◦ Rate of return of family labor
		Socio-cultural criterion / indicators
		• Life quality and human well-being of chain actors & corporate social and ethical responsibility
		  ◦ Companie's voluntary inclusion of social concerns in their activities
		• Women's participation in business production and management
		  ◦ Presence of women in business production and management
		• Social inclusion
		  ◦ Presence of disadvantaged groups in agribusiness
		• Relations with the local community
		  ◦ Collaboration with the local community, local authorities, and civil society
		  ◦ Social capital of agribusinesses
		• Promotion of local identity and transmission of traditional knowledge to the new generations
		  ◦ Activities other than agricultural production as a means for promoting the cultural identity
		  ◦ Preservation of traditions and local culture
		  ◦ Inter generation transmission of traditional knowledge
		• Workers' training planning throughout the chain
		  ◦ Workers' training throughout the chain
		• Implementation of training and foreign labor inclusion programs
		  ◦ Inclusion and training of foreign workers
		• Respect for animal welfare
		  ◦ Application of measures of animal welfare
		Nutrition-health criterion / indicators
		• Healthiness and food safety
		  ◦ Farm distinctiveness
		  ◦ Nutritional quality of solid agro-food material
		  ◦ Nutritional quality of liquid agro-food material
		  ◦ Nutritional quality by food groups (Critical nutrients, whose concentrations are considered for calculating the macro-indicator on the nutritional quality for each group of foods. For details see original publication)

diversity score. In the "environment" thematic area, the authors proposed as sustainability indicators the food biodiversity composition and consumption, rate of local/regional foods and seasonality, and rate of eco-friendly food production and/or consumption. Proposed indicators for "lifestyle" thematic area were physical activity/physical inactivity prevalence, and adherence to the Mediterranean dietary pattern; while for the "clinical aspects" of the nutritional sustainability, the authors proposed the diet-related morbidity/mortality statistics and nutritional anthropometry as indicators.

Nutritional Sustainability of MedDiet Typical Agro-Food Products

A methodological approach to assess the environmental, economic, socio-cultural, and health-nutrition sustainability of Apulian agro-food products was proposed by Capone et al. (49) in 2016.

Azzini et al., including the authors of the above-mentioned study, the latter published a study (48) on the health-nutrition dimension of the typical agro-food products. Two main aspects of health-nutrition sustainability were considered: (1) the business distinctiveness of agro-food companies and food safety and (2) the nutritional quality of foodstuffs. It is important to mention that this work seems to be a refinement of the indicators identified in the nutrition-health principle published in the work of Capone et al. (49).

The proposed indicators for health-nutrition sustainability are reviewed in **Table 6**. The business distinctiveness aspect refers to farms/companies (company-based approach). It includes indicators that are not specific to a single product and depend on the whole management of the agro-food company. To evaluate a company's distinctiveness and food safety, the application of different regulations and standards regarding food safety together with statutory, regulatory, and voluntary requirements, the origins of the raw materials used, and marketing and labeling were considered.

The second aspect, the nutritional quality, refers to each individual product (product-based approach). The nutritional quality of products was assessed taking into consideration their crucial nutrient content, these nutrients being specific for each food product category/group. The selection criteria for nutritional indicators in the nutritional quality aspect were based on secondary data from scientific literature and other relevant sources. The authors considered "bioactive compound" biomarkers, present in foodstuff, in relation to their effect on the health of individuals and groups.

DISCUSSION

This is the first scoping review of the methodological assessment of MedDiet nutritional sustainability. A previous study (18) systematically reviewed the studies on sustainable diets to identify the components of sustainability that were measured and the methods applied to do so. In this work, we reviewed the scientific literature to identify the main components of the nutritional sustainability of MedDiet and the methods that have been applied to assess those components. The concept of nutritional sustainability is broad and complex and encompasses the three dimensions of sustainability, environmental, economic, and socio-cultural, and also the health-nutrition dimension (8).

Through our search strategy, we identified 28 articles; 24 studies exploring the dimensions of nutritional sustainability of the MedDiet (22–45), and 4 proposing the methodological approaches to assess the nutritional sustainability of MedDiet (46, 47) or the sustainability of typical agro-foods of MedDiet (48, 49). From the 24 studies exploring the sustainability of MedDiet, none fully addressed the complexity of the four dimensions of nutritional sustainability (environmental, economic, socio-cultural, and health-nutrition). One of the methodological proposals to assess the nutritional sustainability of MedDiet (47) contemplated the four dimensions of nutritional sustainability, as well as one of the methodological proposals to assess the sustainability of typical agro-foods of MedDiet (49). Nevertheless, no study was identified, through our search strategy or through the list of citing articles, applying those methodological proposals. The remaining methodological proposals (46, 48) were further characterizations of the health-nutrition dimension of sustainability from the previously mentioned studies.

From the research articles, several sustainability indicators were identified. Most of the identified research articles reported sustainability indicators pertaining to the environmental dimension of nutritional sustainability (23–29, 31–45). Six studies (22, 26, 33, 40, 41, 44) reported economic sustainability indicators and six studies (26, 30, 34, 37, 42, 43) reported the sustainability indicators of the health-nutrition dimension of nutritional sustainability. Two studies used indices that combined indicators from the environmental and health-nutrition components of sustainability (26, 42). No studies have reported indicators regarding the socio-cultural dimension. These results are not surprising, due to the large attention that the environmental dimension of sustainability has received over time and are in line with the results obtained in the systematic review of Jones et al. (18) where environmental indicators were reported in most of the identified studies; substantial less studies reported economic sustainability indicators and indicators of the socio-cultural dimension, such as the examination of cultural heritage and skills, equity, and rights, were almost entirely lacking.

Two of the leading threats to global health are climate change and non-communicable diseases, both of which are inextricably linked to diet (20, 50); in this sense, nutritional sustainability goes along with the One Health concept where human, animal, and the environmental health are intimately linked (51). The One Health approach, by definition, encompasses many fields, including, but not limited to, health, ecology, agriculture and sustainability, economics, anthropology, and the social sciences (52). All those disciplines are also included in the assessment of nutritional sustainability. Assessing the environmental dimension of sustainability is of utmost importance. The emissions of the global food system (from food production to consumption) are estimated to account for 21–37% of total human-induced GHGs emissions, 70% of freshwater use, increased eutrophication, and consumption of 35% of ice-free land, and it is also the greatest cause of deforestation and biodiversity loss, thereby contributing to the detrimental effects on natural resources (19, 24, 53). Recently, the report of the EAT-Lancet Commission on healthy diets from sustainable food systems (1) indicated that food systems are the major driver of environmental degradation and further food production should use no additional land, safeguard existing biodiversity, reduce consumptive water use and manage water responsibly, substantially reduce nitrogen and phosphorus pollution, produce zero carbon dioxide emissions, and cause no further increase in methane and nitrous oxide emissions. Sustainability indicators to assess those recommendations were found in the articles included in this review. Among the indicators cited, the most used were related to global warming potential (GHGs emissions and CF) (23–29, 31, 33, 35–37, 40, 41, 43–45), followed by water (25–29, 31–33, 35, 38, 39, 42, 44, 45), land (23, 25, 26, 29, 31, 35, 37, 43, 45), and energy use (23, 25–29, 40, 45). Our findings are in line with the previous studies where the global warming potential of diets was by far the most commonly measured environmental sustainability indicator, with land, energy, and water use also frequently assessed (18). Considering the detrimental impacts that food systems have on the environment, it is not surprising to observe the abundance of those sustainability indicators in the identified literature. Most of the studies used the life cycle assessment (LCA) approach to obtain environmental sustainability indicators. This finding is consistent with the literature on the subject, where LCA is the most commonly used approach (18–20, 54). Despite being the most commonly used approach, LCA methodology is not free from limitations (55), and other methodologies to assess sustainability, such as the modeling approaches, integrated analytical frameworks, and the proposed adaptive, participatory methods, have been proposed (18).

From the environmental perspective, many of the identified studies consistently found that MedDiet is a sustainable option (25–31, 33, 38–40, 42–45). Nevertheless, some studies relying on dietary consumption data or dietary scenarios reported that in some cases, other dietary patterns had a similar or better environmental performance (22, 25, 26, 28, 32, 34–37, 41), while the mixed studies, based on dietary consumption and dietary scenarios, indicated MedDiet as the most environmentally friendly option (42–45). Studies examining the impact of foods on environmental sustainability reported animal food sources as the food category with the most deleterious environmental effects (25, 34, 35). As previously mentioned, MedDiet is a dietary pattern characterized by moderate consumption of eggs, poultry, and dairy products (cheese and yogurt) and low consumption of red meat (13, 14). Furthermore, in its present update, the MedDiet pyramid reflected multiple environmental concerns and strongly emphasizes a lower consumption of red meat and bovine dairy products (13, 56).

Six studies (22, 26, 33, 40, 41, 44) measured the cost associated with the adherence to MedDiet as a measure of economic sustainability. Those studies shed some light on the economic tradeoffs of adhering to MedDiet. In two of the studies (26, 44), adherence to the MedDiet, compared to other patterns of dietary consumption, was associated with a higher cost; yet, in one study (33), it was proposed that isocaloric diets have approximately the same cost. These results may be explained by the different methodological approaches used in each study but are most likely explained by the dietary patterns compared to the MedDiet. The MedDiet was more expensive than the Western dietary pattern and the Provegetarian dietary pattern (26), slightly more expensive than the dietary consumption of the Italian population (44); no significant differences were observed between the MedDiet, the SEAD, and the NAOS (33). Monetary cost is one of the key factors in food choice and it is the main factor in shaping the consumer demand; therefore, it will affect consumer preferences and options for a sustainable dietary pattern (18, 57). Food prices condition the affordability of sustainable diets. Low prices reduce the income of producers, reduce their ability to invest, and may hinder the development of a sustainable food system. From the sustainability point of view, price is ambivalent; therefore, it is important to guarantee the accessibility and affordability to food choices in order to ensure economic sustainability but at the same time, the affordability may have negative environmental impacts by not discouraging food waste (58). In line with our findings, there is evidence indicating that MedDiet is not necessarily associated with higher overall dietary costs (59).

The health-nutrition dimension of nutritional sustainability of MedDiet was assessed in six studies (26, 30, 34, 37, 42, 43). Fresán et al. (26) used the advantage of a longitudinal study to explore the time by which a rate of an outcome (death, non-fatal cardio vascular disease (CVD) myocardial infarction or stroke, non-fatal breast cancer, or type 2 diabetes mellitus, whichever occurred first) is advanced or is postponed within individuals exposed to different dietary patterns. The NRF9.3 and NQI were also used to assess the diet quality in combination with FF, to quantify the satiety response of food (34). van Dooren et al. (37, 43) used a health score, that was composed by the ratio between the consumption and the recommendations for some food groups, nutrients, and energy. Regardless of the methodological differences, MedDiet was associated with a better performance in the health-nutrition dimension. MedDiet has been consistently shown to be a healthy dietary pattern that may reduce risk related to non-communicable diseases (60); and therefore, adherence to the MedDiet or other healthy dietary patterns may be associated with the sustainability of healthcare systems.

The absence of exploration regarding the socio-cultural dimension of sustainability in the identified literature is particularly important, given the critical role of society and culture in the MedDiet. The relevance of this dimension is so clear that MedDiet was acknowledged by UNESCO as an intangible cultural heritage (61). According to UNESCO, MedDiet is a way of life that encompasses a set of skills, knowledge, rituals, symbols, and traditions, ranging from landscape to the table. Eating together is the foundation of the cultural identity and continuity of communities throughout the Mediterranean Basin. The MedDiet emphasizes values of hospitality, neighborliness, intercultural dialogue and creativity, and a way of life guided by respect for diversity (17). Despite its increasing popularity worldwide, adherence to the MedDiet is decreasing due to multifactorial influences, such as globalization, population growth, and socio-economic changes. Food chain modernization has increased productivity and resulted in a substantial transformation of lifestyles as a consequence of rising incomes, urbanization, and changes in the agricultural and food sectors. Those changes threaten seriously the transmission and preservation of the MedDiet heritage to present and future generations (17). Measuring the sustainability of the socio-cultural dimension is paramount for the preservation of MedDiet.

Six studies (23, 25, 26, 37, 42, 43, 51) combined indicators to provide a "sustainability index." Most of the studies combined environmental indicators into environmental sustainability indices (23, 25, 37, 43). Fresán et al. (26) designed an index that gathered the impact of the daily diet on the analyzed aspects: health, environmental footprints, and monetary costs. Blas et al. (42) proposed the nutritional water productivity (NWP) that links water and nutrition. The development of indices that combine all the dimensions of nutritional sustainability may facilitate its assessment and the comparability of different dietary patterns or food products.

We did not identify studies that used methodological approaches covering all the conceptual framework of nutritional sustainability of MedDiet; instead, we identified studies that assessed some dimensions of MedDiet nutritional sustainability. Heterogeneity in the indicators used was found, particularly in the environmental dimension. Studies on the economic and health-nutrition dimensions are less frequent and absent in the socio-cultural dimension. Our findings call for the development of harmonized methodologies for the assessment of MedDiet nutritional sustainability. Indeed, the methodological approach proposed by Dernini et al. (47) identified indicators to assess the sustainability of the four dimensions that should be considered. Despite being comprehensive and complete, no indication is given regarding the weight of each dimension or the indicator for a sustainability score; although the authors mention that the methodological approach requires to be tested and further refined in a group of selected Mediterranean countries, indicating that this is an ongoing work.

Traditional and typical agro-food products are at the core of MedDiet (49). A typical agro-food product is characterized by historical and cultural features and by physical attributes that are deep-rooted to the territory of origin encompassing much more than organoleptic qualities. In the last years, we have observed a deep transformation in consumer perception and in the demand for typical agro-food products. The retrieval of typical and traditional foods represents an attempt to recover the safety and social aspects of eating habits. To form positive attitudes and expectations toward food, consumers need to be assured and informed about the production and transformation processes as well as about their origin and the symbolic values they encompass (62). Typical agro-food products contribute directly and indirectly to the sustainability of the MedDiet in the Mediterranean basin (49). Considering those aspects, we identified two works related to the sustainability of typical agro-food products (48, 49). Capone et al. (49) proposed a comprehensive approach to assess the sustainability of typical agro-food products of the MedDiet. This methodological proposal englobes all the dimensions of sustainability that are explored in our study. The identified work of Azzini et al. (48) seems to provide clarification to the health-nutrition dimension mentioned in the work of Capone et al. (49).

In this work, sustainability was assessed in the environmental, economic, sociocultural, and health-nutrition dimensions. Considering the included literature, environmental sustainability was assessed and defined as the ability to use fewer resources (23, 25–29, 31–33, 35, 37–40, 42–45) to produce less byproducts (23–29, 31, 33–37, 40, 41, 43–45). Economic sustainability was defined as the ability to promote economic growth (41) or the accessibility to the consumers (22, 26, 33, 40, 44). The Heath-nutrition dimension was defined as the capability to provide adequate nutrition (30, 37, 42, 43), promote health, and prevent disease (26). Despite not being assessed, the socio-cultural dimension of sustainability encompasses historical remains and values, local culture, and traditions; therefore, it was defined as the ability to preserve them (63). Nutritional sustainability is an umbrella term that can take several meanings depending on the dimension that is assessed.

Several considerations must be made regarding the findings of this study. Most of the studies identified are from the countries located in the Mediterranean basin and the remaining are from Northern Europe and the United States. While it is not surprising to find studies regarding MedDiet sustainability in the countries of its origin, MedDiet is recommended worldwide as a sustainable dietary option (64); therefore, studies on other regions are needed. Comparisons are difficult due to the heterogeneity of the indicators used in the identified studies and no studies used a comprehensive approach that explores nutritional sustainability in all dimensions. Harmonization is essential for the comparison of results; yet, a significant degree of flexibility is also needed to allow for the wide application of an instrument to assess the nutritional sustainability of diets or food products that are, by nature, dynamic. Identified studies did not provide examples of approaches to combine all the indicators of sustainability. Identified articles were published between 2012 and 2021, highlighting the recent interest in the subject. Despite a significant body of literature that meets the inclusion criteria for this review, more work is needed to establish a consensual approach to assess the nutritional sustainability of MedDiet and to compare it with other dietary patterns.

Our scoping review has some limitations. A search was performed only in two electronic databases (Scopus and PubMed); therefore, relevant works may have been missed. Gray literature could be an informative source of evidence to this study; however, the sizable amount of gray literature in the field could have dumped the feasibility of the work. The search strategy was broad enough to capture a significant body of literature in the area, yet it is possible that studies assessing the sustainability indicators but not mentioning the word sustainability (or related words) have not been captured.

Our study reviewed for the first time the assessment of the nutritional sustainability of MedDiet. From a general perspective, there is sufficient evidence to state that MedDiet is a nutritional sustainable option. Methodological assessment of nutritional sustainability is challenging and involves multidisciplinary approaches. To the best of our knowledge, no research has been made assessing MedDiet in all the dimensions of the complex concept, that is nutritional sustainability. In its concept, nutritional sustainability is differentiated from other concepts combining nutrition and sustainability; it does not contradict with other similar concepts (sustainable diet and sustainable food systems) but aggregates concepts from them. MedDiet nutritional sustainability needs to attract sufficient political attention and become a core priority in the shaping of agriculture, food, and nutrition policies; for that, research needs, in a comprehensive way, to reflect the complexity of the nutritional sustainability concept. Integrating health and nutrition, environmental, economic, and socio-cultural considerations across scales and contexts can offer a more complete understanding of the opportunities and barriers to achieving nutritional sustainability not only in MedDiet but also in other dietary patterns and food products.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

CP-N wrote the first draft of the manuscript. The data acquisition of the article and analysis of its content has been made by a consensus between CP-N and CG. CS and CG conceived and designed the study. All the authors had revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.772133/full#supplementary-material

REFERENCES

1. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the anthropocene: the EAT-*Lancet* commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4
2. Lang T, Barling D. Nutrition and sustainability: an emerging food policy discourse. *Proc Nutr Soc.* (2013) 72:1–12. doi: 10.1017/S002966511200290X
3. Gussow JD, Clancy KL. Dietary guidelines for sustainability. *J Nutr Educ.* (1986) 18:1–5. doi: 10.1016/S0022-3182(86)80255-2
4. Burlingame B, Dernini S. *Sustainable Diets And Biodiversity Directions And Solutions For Policy, Research And Action*. Rome: FAO Headquarters (2012).
5. Timmermans A, Ambuko J, Belik W, Huang J. *Food Losses And Waste In The Context Of Sustainable Food Systems.* CFS Committee on World Food Security HLPE (2014).
6. Meybeck A, Redfern S, Paoletti F, Strassner C. *Assessing Sustainable Diets Within the Sustainablility of Food Systems*. Mediterranean diet, organic food: new chalenges FAO, Rome. (2014).
7. Swanson KS, Carter RA, Yount TP, Aretz J, Buff PR. Nutritional sustainability of pet foods. *Adv Nutr.* (2013) 4:141–50. doi: 10.3945/an.112.003335
8. Smetana SM, Bornkessel S, Heinz V. A path from sustainable nutrition to nutritional sustainability of complex food systems. *Front Nutr.* (2019) 6:39. doi: 10.3389/fnut.2019.00039
9. Burlingame B, Dernini S. Sustainable diets: the mediterranean diet as an example. *Public Health Nutrition.* (2011) 14:2285–7. doi: 10.1017/S1368980011002527
10. Dernini S, Berry EM. Mediterranean diet: from a healthy diet to a sustainable dietary pattern. *Front Nutr.* (2015) 2:15. doi: 10.3389/fnut.2015.00015
11. Berry EM. Sustainable food systems and the mediterranean diet. *Nutrients.* (2019) 11:2229. doi: 10.3390/nu11092229
12. Medina FX. Food consumption and civil society: mediterranean diet as a sustainable resource for the mediterranean area. *Public Health Nutr.* (2011) 14:2346–9. doi: 10.1017/S1368980011002618
13. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. science and cultural updates. *Public Health Nutr.* (2011) 14:2274–84. doi: 10.1017/S1368980011002515
14. D'Alessandro A, De Pergola G. The mediterranean diet: its definition and evaluation of a priori dietary indexes in primary cardiovascular prevention. *Int J Food Sci Nutr.* (2018) 69:647–59. doi: 10.1080/09637486.2017.1417978
15. Bonaccio M, Iacoviello L, Donati MB, de Gaetano G. The tenth anniversary as a UNESCO world cultural heritage: an unmissable opportunity to get back to the cultural roots of the mediterranean diet. *Eur J Clin Nutr.* (2021). doi: 10.1038/s41430-021-00924-3
16. Dinu M, Pagliai G, Casini A, Sofi F. Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. *Eur J Clin Nutr.* (2018) 72:30–43. doi: 10.1038/ejcn.2017.58
17. Dernini S, Berry EM, Serra-Majem L, La Vecchia C, Capone R, Medina F, et al. Med Diet 40: the mediterranean diet with four sustainable benefits. *Public Health Nutr.* (2017) 20:1322–30. doi: 10.1017/S1368980016003177
18. Jones AD, Hoey L, Blesh J, Miller L, Green A, Shapiro LF, et al. systematic review of the measurement of sustainable diets. *Adv Nutr.* (2016) 7:641–64. doi: 10.3945/an.115.011015
19. Coats L, Aboul-Enein BH, Dodge E, Benajiba N, Kruk J, Khaled MB, et al. Perspectives of environmental health promotion and the mediterranean diet: a thematic narrative synthesis. *J Hunger Environ Nutr.* (2020) 1—23. doi: 10.1080/19320248.2020.1777242
20. Reinhardt SL, Boehm R, Blackstone NT, El-Abbadi NH, McNally Brandow JS, Taylor SF, et al. Systematic review of dietary patterns and sustainability in the United States. *Adv Nutr.* (2020) 11:1016–31. doi: 10.1093/advances/nmaa026
21. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. *Ann Intern Med.* (2018) 169:467–73. doi: 10.7326/M18-0850
22. Llanaj E, Hanley-Cook GT. Adherence to healthy and sustainable diets is not differentiated by cost, but rather source of foods among young adults in Albania. *Br J Nutr.* (2021) 126:591–9. doi: 10.1017/S00071145200 04390
23. Grasso AC, Olthof MR, van Dooren C, Roca M, Gili M, Visser M, et al. Effect of food-related behavioral activation therapy on food intake and the environmental impact of the diet: results from the MooDFOOD prevention trial. *Eur J Nutr.* (2020) 59:2579–91. doi: 10.1007/s00394-019-02106-1
24. Rosi A, Biasini B, Donati M, Ricci C, Scazzina F. Adherence to the mediterranean diet and environmental impact of the diet on primary school

children living in parma (Italy). *Int J Environ Res Public Health.* (2020) 17:6105. doi: 10.3390/ijerph17176105

25. Grosso G, Fresán U, Bes-Rastrollo M, Marventano S, Galvano F. Environmental impact of dietary choices: role of the mediterranean and other dietary patterns in an italian cohort. *Int J Environ Res Public Health.* (2020) 17:1468. doi: 10.3390/ijerph17051468
26. Fresán U, Martínez-González MA, Sabaté J, Bes-Rastrollo M. Global sustainability (health, environment and monetary costs) of three dietary patterns: results from a Spanish cohort (the SUN project). *BMJ Open.* (2019) 9:e021541. doi: 10.1136/bmjopen-2018-021541
27. Naja F, Itani L, Hamade R, Chamieh MC, Hwalla N. Mediterranean diet and its environmental footprints amid nutrition transition: the case of Lebanon. *Sustainability.* (2019) 11:6690. doi: 10.3390/su11236690
28. Naja F, Jomaa L, Itani L, Zidek J, El Labban S, Sibai AM, et al. Environmental footprints of food consumption and dietary patterns among Lebanese adults: a cross-sectional study. *Nutr J.* (2018) 17:85. doi: 10.1186/s12937-018-0393-3
29. Fresán U, Martínez-Gonzalez MA, Sabaté J, Bes-Rastrollo M. The mediterranean diet, an environmentally friendly option: evidence from the seguimiento universidad de navarra (SUN) cohort. *Public Health Nutr.* (2018) 21:1573–82. doi: 10.1017/S1368980017003986
30. Seconda L, Baudry J, Allès B, Hamza O, Boizot-Szantai C, Soler L-G, et al. Assessment of the sustainability of the mediterranean diet combined with organic food consumption: an individual behaviour approach. *Nutrients.* (2017) 9:61. doi: 10.3390/nu9010061
31. Belgacem W, Mattas K, Arampatzis G, Baourakis G. Changing dietary behavior for better biodiversity preservation: a preliminary study. *Nutrients.* (2021) 13:2076. doi: 10.3390/nu13062076
32. Vanham D, Guenther S, Ros-Baró M, Bach-Faig A. Which diet has the lower water footprint in Mediterranean countries? *Resour Conserv Recycl.* (2021) 171:105631. doi: 10.1016/j.resconrec.2021.105631
33. González-García S, Green RF, Scheelbeek PF, Harris F, Dangour AD. Dietary recommendations in Spain –affordability and environmental sustainability? *J Clean Prod.* (2020) 254:120125. doi: 10.1016/j.jclepro.2020.120125
34. Chapa J, Farkas B, Bailey RL, Huang J-Y. Evaluation of environmental performance of dietary patterns in the United States considering food nutrition and satiety. *Sci Total Environ.* (2020) 722:137672. doi: 10.1016/j.scitotenv.2020.137672
35. Blackstone NT, El-Abbadi NH, McCabe MS, Griffin TS, Nelson ME. Linking sustainability to the healthy eating patterns of the dietary guidelines for Americans: a modelling study. *Lancet Planet Health.* (2018) 2:e344–e52. doi: 10.1016/S2542-5196(18)30167-0
36. Ulaszewska MM, Luzzani G, Pignatelli S, Capri E. Assessment of diet-related GHG emissions using the environmental hourglass approach for the mediterranean and new Nordic diets. *Sci Total Environ.* (2017) 574:829–36. doi: 10.1016/j.scitotenv.2016.09.039
37. van Dooren C, Aiking H. Defining a nutritionally healthy, environmentally friendly, and culturally acceptable Low Lands Diet. *Int J Life Cycle Assess.* (2016) 21:688–700. doi: 10.1007/s11367-015-1007-3
38. Vanham D, del Pozo S, Pekcan AG, Keinan-Boker L, Trichopoulou A, Gawlik BM. Water consumption related to different diets in mediterranean cities. *Sci Total Environ.* (2016) 573:96–105. doi: 10.1016/j.scitotenv.2016.08.111
39. Blas A, Garrido A, Willaarts BA. Evaluating the water footprint of the mediterranean and American diets. *Water.* (2016) 8:448. doi: 10.3390/w8100448
40. Pairotti MB, Cerutti AK, Martini F, Vesce E, Padovan D, Beltramo R. Energy consumption and GHG emission of the mediterranean diet: a systemic assessment using a hybrid LCA-IO method. *J Clean Prod.* (2015) 103:507–16. doi: 10.1016/j.jclepro.2013.12.082
41. Rahmani R, Bakhshoodeh M, Zibaei M, Heijman W, Eftekhari MH. Economic and environmental impacts of dietary changes in Iran: an input-output analysis. *Int J Food Syst Dyn.* (2011) 2:447–63. doi: 10.18461/ijfsd.v2i4.248
42. Blas A, Garrido A, Unver O, Willaarts B. A comparison of the mediterranean diet and current food consumption patterns in Spain from a nutritional and water perspective. *Sci Total Environ.* (2019) 664:1020–9. doi: 10.1016/j.scitotenv.2019.02.111
43. van Dooren C, Marinussen M, Blonk H, Aiking H, Vellinga P. Exploring dietary guidelines based on ecological and nutritional values: a comparison of six dietary patterns. *Food Policy.* (2014) 44:36–46. doi: 10.1016/j.foodpol.2013.11.002
44. Germani A, Vitiello V, Giusti AM, Pinto A, Donini LM, del Balzo V. Environmental and economic sustainability of the mediterranean diet. *Int J Food Sci Nutr.* (2014) 65:1008–12. doi: 10.3109/09637486.2014.945152
45. Sáez-Almendros S, Obrador B, Bach-Faig A, Serra-Majem L. Environmental footprints of Mediterranean versus Western dietary patterns: beyond the health benefits of the Mediterranean diet. *Environ Health.* (2013) 12:118. doi: 10.1186/1476-069X-12-118
46. Donini LM, Dernini S, Lairon D, Serra-Majem L, Amiot M-J, del Balzo V, et al. A Consensus proposal for nutritional indicators to assess the sustainability of a healthy diet: the mediterranean diet as a case study. *Front Nutr.* (2016) 3:37. doi: 10.3389/fnut.2016.00037
47. Dernini S, Meybeck A, Burlingame B, Gitz V, Lacirignola C, Debs P, et al. Developing a methodological approach for assessing the sustainability of diets: the mediterranean diet as a case study. *New Medit.* (2013) 12:28–37.
48. Azzini E, Maiani G, Turrini A, Intorre F, Lo Feudo G, Capone R, et al. The health-nutrition dimension: a methodological approach to assess the nutritional sustainability of typical agro-food products and the mediterranean diet. *J Sci Food Agric.* (2018) 98:3684–705. doi: 10.1002/jsfa.8877
49. Capone R, Bilali HE, Bottalico F. Assessing the sustainability of typical agro-food products: insights from apulia region, Italy. *New Medit.* (2016) 15:28–35.
50. Scheres J, Kuszewski K. The ten threats to global health in 2018 and 2019. a welcome and informative communication of WHO to everybody. *Public Health Management/Zdrowie Publiczne i Zarzadzanie.* (2019) 17:2–8. doi: 10.4467/20842627OZ.19.001.11297
51. van Helden PD, van Helden LS, Hoal EG. One world, one health. *EMBO Rep.* (2013) 14:497–501. doi: 10.1038/embor.2013.61
52. Davis MF, Rankin SC, Schurer JM, Cole S, Conti L, Rabinowitz P, et al. Checklist for one health epidemiological reporting of evidence (COHERE). One *Health.* (2017) 4:14–21. doi: 10.1016/j.onehlt.2017.07.001
53. Hallström E, Carlsson-Kanyama A, Börjesson P. Environmental impact of dietary change: a systematic review. *J Clean Prod.* (2015) 91:1–11. doi: 10.1016/j.jclepro.2014.12.008
54. Eme PE, Douwes J, Kim N, Foliaki S, Burlingame B. Review of methodologies for assessing sustainable diets and potential for development of harmonised indicators. *Int J Environ Res Public Health.* (2019) 16:1184. doi: 10.3390/ijerph16071184
55. Finnveden G. On the limitations of life cycle assessment and environmental systems analysis tools in general. *Int J Life Cycle Assess.* (2000) 5:229. doi: 10.1007/BF02979365
56. Serra-Majem L, Tomaino L, Dernini S, Berry EM, Lairon D, Ngo de la Cruz J, et al. Updating the mediterranean diet pyramid towards sustainability: focus on environmental concerns. *Int J Environ Res Public Health.* (2020) 17:8758. doi: 10.3390/ijerph17238758
57. Hoek AC, Pearson D, James SW, Lawrence MA, Friel S. Healthy and environmentally sustainable food choices: Consumer responses to point-of-purchase actions. *Food Qual Prefer.* (2017) 58:94–106. doi: 10.1016/j.foodqual.2016.12.008
58. Meybeck A, Gitz V. Sustainable diets within sustainable food systems. *Proc Nutr Soc.* (2017) 76:1–11. doi: 10.1017/S0029665116000653
59. Drewnowski A, Eichelsdoerfer P. The Mediterranean diet: does it have to cost more? *Public health nutr.* (2009) 12:1621–8. doi: 10.1017/S1368980009990462
60. Martinez-Lacoba R, Pardo-Garcia I, Amo-Saus E, Escribano-Sotos F. Mediterranean diet and health outcomes: a systematic meta-review. *Eur J Public Health.* (2018) 28:955–61. doi: 10.1093/eurpub/cky113
61. Trichopoulou A. Mediterranean diet as intangible heritage of humanity: 10 years on. *Nutr Metab Cardiovasc Dis.* (2021) 31:1943–8. doi: 10.1016/j.numecd.2021.04.011
62. Nosi C, Zanni L. Moving from “typical products” to “food-related services”. *Br Food J.* (2004) 106:779–92. doi: 10.1108/00070700410561388
63. Axelsson R, Angelstam P, Degerman E, Teitelbaum S, Andersson K, Elbakidze M, et al. Social and cultural sustainability: criteria, indicators, verifier variables for measurement and maps for visualization to support planning. *Ambio.* (2013) 42:215–28. doi: 10.1007/s13280-012-0376-0
64. Garcia-Alvarez-Coque J-M, Abdullateef O, Fenollosa L, Ribal J, Sanjuan N, Soriano JM. Integrating sustainability into the multi-criteria

assessment of urban dietary patterns. *Renew Agric Food Syst.* (2021) 36:69–76. doi: 10.1017/S174217051900053X

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Leveraging Digital Tools and Crowdsourcing Approaches to Generate High-Frequency Data for Diet Quality Monitoring at Population Scale in Rwanda

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Diet quality is a critical determinant of human health and increasingly serves as a key indicator for food system sustainability. However, data on diets are limited, scattered, often project-dependent, and current data collection systems do not support high-frequency or consistent data flows. We piloted in Rwanda a data collection system, powered by the principles of citizen science, to acquire high frequency data on diets. The system was deployed through an unstructured supplementary service data platform, where respondents were invited to answer questions regarding their dietary intake. By combining micro-incentives with a normative nudge, 9,726 responses have been crowdsourced over 8 weeks of data collection. The cost per respondent was <$1 (system set-up, maintenance, and a small payment to respondents), with interactions taking <15 min. Exploratory analyses show that >70% of respondents consume tubers and starchy vegetables, leafy vegetables, fruits, legumes, and wholegrains. Women consumed better quality diets than male respondents, revealing a sex-based disparity in diet quality. Similarly, younger respondents (age ≤24 years) consumed the lowest quality diets, which may pose significant risks to their health and mental well-being. Middle-income Rwandans were identified to have consumed the highest quality diets. Long-term tracking of diet quality metrics could help flag populations and locations with high probabilities of nutrition insecurity, in turn guiding relevant interventions to mitigate associated health and social risks.

Keywords: citizen science, dietary patterns, digital data collection, USSD, Rwanda

INTRODUCTION

The triple burden of malnutrition: undernourishment, micronutrient deficiencies, and over nutrition is a global challenge, with almost a billion people experiencing undernutrition and a further 2 billion currently overweight (Global Nutrition Report Independent Expert Group, 2020). Malnutrition has been linked to a multitude of factors, including socio-economic

(Reinhardt and Fanzo, 2014), with undernutrition often associated with lower and middle-income countries and overnutrition with high income countries. However, this spectrum is increasingly blurred, with the different forms of malnutrition now observable in the same country, household, and even in the same person (Doak et al., 2000, 2005; Global Nutrition Report Independent Expert Group, 2018).

In low-income countries, consumption of healthy foods is in decline, with simultaneous increases in unhealthy foods (Imamura et al., 2015). In lower-middle income countries (LMICs), increased consumption of both healthy and unhealthy foods has been observed (Imamura et al., 2015). These dynamic patterns are in many cases the manifestation of nutrition transitions, where wealthier and more urban individuals shift towards consumption of processed, sweetened, and salted foods (Popkin, 2015). In Africa, younger, increasingly urban, and wealthier populations have experienced major nutrition transitions over recent decades (Vorster et al., 2011; Steyn and McHiza, 2014; Kinyonki et al., 2020). In Rwanda, for example, 33% of the population remains undernourished (National Institute of Statistics of Rwanda and ICF, 2020), with nutritionally inadequate diets widely prevalent (Marivoet et al., 2020; Arsenault and Olney, 2021). Like many other LMICs however, nutrition transitions are evident in Rwanda, with people moving away from traditional plant-based diets of leafy vegetables, fruits, legumes, and grains. A consequence of such a transition is that the population of overweight children is larger than that suffering wasting (FAO, 2018; World Bank, 2019; Kinyonki et al., 2020).

Global malnutrition and dietary transitions reinforce the need to promote innovative and nutrition-focused food systems (Gómez et al., 2013). To do so, Popkin et al. (2020) argue that new ways of designing and targeting nutrition programmes and policies are needed. These programmes would require concerted efforts to improve the availability, access, frequency, and quality of data on diets. Unfortunately, government and non-government agencies in LMICs generally do not have data collection systems needed for concerted high frequency data collection (Tuffrey and Hall, 2016). When available, data on diets are from small, fragmented, and temporally static samples and globally modelled data diverge dependent on the source and models used and are therefore limited in terms of actionability for program design (Beal et al., 2021).

To address this, innovative systems and tools for dietary data collection are required that provide basic functionality to engage users, whilst having a back-end that can aggregate diet data and generate insights about dietary patterns across groups. Although diet quality tracking software are widely available for smartphone users (e.g., Ferrara et al., 2019). Unfortunately, similar applications are limited for simple phone (non-smartphone) users, which dominate mobile phone ownership outside of advanced economies (Pew Research Center, 2019). Despite this, contextually sensitive technologies and methods exist (e.g., unstructured supplementary service data, interactive voice response, and computer assisted telephone interviews) to generate a sustained and high-frequency data flow from crowd-based data collection systems (Gibson et al., 2017; Lamanna et al., 2019). The harmonisation of these data collection systems with rapid and globally validated diet quality assessment tools (e.g., Herforth et al., 2020a,b), could provide an opportunity for developing and testing a system for high-frequency diet tracking and rapid diet quality assessment. Construction and testing of a crowd-based system constitutes an indispensable and promising step towards robust data-driven policies and interventions for food and nutrition security (e.g., Wells et al., 2019).

We aim to develop a generic and widely applicable data collection system that leverages on the application of citizen science and digital tools for high frequency collection of diet data. Specifically, we look to provide learning from the development, testing, and application of this system in Rwanda, whilst providing provisional results on the trends in diet quality across Rwandan society. The goal of this system being to generate a robust system that is easily adapted to national dietary and technological conditions to generate insights on diet quality to support evidenced-based policy making and interventions.

METHODS

Study Area

The piloting of this system was conducted in Rwanda (**Figure 1**), a Central African country of around 13 million people. The Rwandan population is largely below 40 years old (National Institute of Statistics Rwanda, 2021). In 2018, at least 17% of the population experienced moderate or severe food insecurity (National Institute of Statistics Rwanda, 2021). In 2016, 56% of the population was living below the international poverty line (National Institute of Statistics Rwanda, 2021), with median household incomes of $2.57 (World Bank, 2021a). Mobile phone penetration is high, with around 76 mobile phone subscriptions per 100 inhabitants (Rwanda Utilities Regulatory Authority, 2020), with 3G mobile network being accessible to 94% of the population (Rwanda Utilities Regulatory Authority, 2019).

Diet Quality Questionnaire

To generate insights on diet quality across Rwanda, we applied the Diet Quality Questionnaire (DQ-Q) (Herforth et al., 2020a). The DQ-Q is already being applied globally, with the Gallup Polling organisation including it in diet module of their global polling (Herforth et al., 2020b).

The DQ-Q captures consumption information for 26 food groups (including both healthy and unhealthy food items) by asking 29 binary questions. The DQ-Q requires respondents to answer either "yes" or "no" to whether they consumed, during the past 24 h, example food items presented in the corresponding question. For more information on the DQ-Q and its global application, please visit: https://www.globaldietquality.org/.

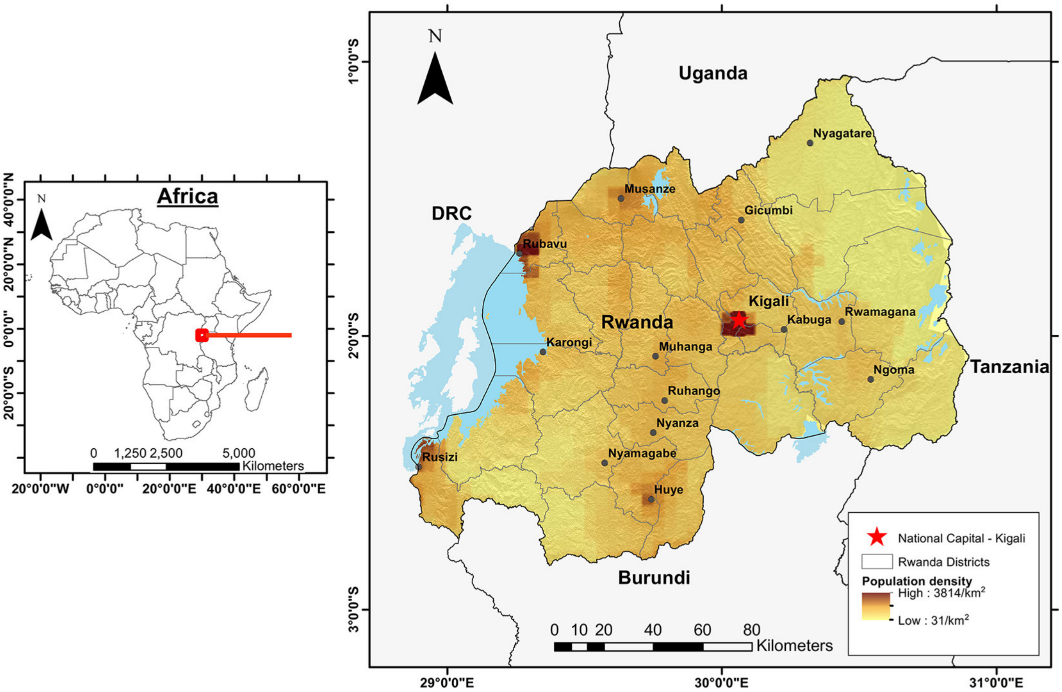

FIGURE 1 | Map of Rwanda. Rwandan districts and population density included for reference.

To make the DQ-Q contextually relevant to Rwanda, it was adapted to national dietary conditions through identification of recognisable and widely available food items for each food group. To do this, we collaborated with the developers of DQ-Q and local governmental authorities. The DQ-Q was translated to Kinyarwanda (Rwanda's official language). The full questionnaire (in English and Kinyarwanda) is available in **Supplementary Material 1**.

From the outputs of the DQ-Q, 12 indicators can be generated. The indicators reflect global dietary recommendations from international authorities (**Table 1**). These indicators were validated by Herforth et al. (2020a) against equivalent quantitative amounts to align with these global dietary recommendations. The outputs of DQ-Q can also be aggregated into three summary indicators (Global Dietary Recommendation score, Global Dietary Recommendation—Healthy score, and Global Dietary Recommendation—Limit score) which are constructed through the presence of relevant food groups (**Table 1**). For further information on the construction and validation of the indicators see Herforth et al. (2020a).

Platform Design

We deployed the DQ-Q collection system using unstructured supplementary service data (USSD). USSD is a free, text-based protocol that allows for two-way exchanges of information (up to 140 characters in Rwanda). The text-based nature of USSD offers flexibility for deployment on both feature and smartphones and deployment in (almost) any country with mobile phone coverage. An example of the USSD-based DQ-Q is shown in **Figure 2**. The system was developed and maintained by the social enterprise VIAMO (www.viamo.io), who have extensive background in developing mobile phone-based systems in LMICs, including Rwanda.

Sampling

Potential respondents for the DQ-Q were sourced from a toll-free information service database (*845#), maintained by VIAMO in collaboration with MTN Rwanda (a telecommunications company). The information service has been live for more than 4 years and provides information on agriculture, health, finance, legal aid, nutrition, youth, news, and weather. It can be accessed by all MTN Rwanda customers who have 8 free calls to the service each month.

The 845-service database contains prospective respondents' mobile number, age, and sex. This information was collected during a voluntary registration process for the service, not related to this project. The information for more than 1.2 million verified individuals (∼9% of the Rwandan population) are held in the database, with around 80% consenting to be contacted for further information or to participate in surveys. Respondents from all districts (sub-national level—administrative level 2) of Rwanda are present in the database.

To track spatial and temporal trends of diet quality, an initial schedule of 28 weekly data collections was developed starting in early August 2021. Data will be collected until February 2022. A representative sample of ∼2,500 respondents for each weekly

TABLE 1 | Diet quality questionnaire indicators.

Indicator	WHO dietary recommendation*	Description	Food group components	Score
Fruits and vegetables	>400 g	Score based upon the presence of up to 6 fruit and vegetable groups in diets	Dark green leafy vegetables Vitamin-A rich orange-coloured vegetables, roots, and tubers Other vegetables Vitamin A-rich fruits Citrus fruits Other fruits	0–6
Dietary fibre	>25 g	Score based upon the presence of 9 food groups rich in dietary fibre	Dark green leafy vegetables Vitamin-A rich orange-coloured vegetables, roots, and tubers Other vegetables Vitamin A-rich fruits Citrus fruits Other fruits Legumes (double weighted) Nuts and seeds Whole grains	0–10
Free sugars	<10% total energy	Score based upon presence of up to 6 groups of sugary foods	Sodas Baked grains Other sweets Fruit drinks Sweetened drinks	0–5
Saturated fat	<10% total energy	Score based upon presence of 5 food groups rich in saturated fats in diets	Cheese and yoghurt Processed meat (double weighted) Unprocessed red meat Other sweets Fast food Milk	0–6
Total fat	<30% total energy	Score based upon presence of 7 food groups rich in fat	Cheese and yoghurt Processed meat Unprocessed red meat Deep-fried food Fast food Packaged salty snacks Other sweets	0–7
Legumes	>0 g	Scored based upon the presence of legumes in diets	Legumes	0–1
Nuts and seeds	>0 g	Scored based upon the presence of nuts and seeds in diets	Nuts and seeds	0–1
Whole grains	>0 g	Scored based upon the presence of whole grains in diets	Whole grains	0–1
Processed meat	0 g	Scored based upon the presence of processed meats in diets	Processed meat	0–1
Global dietary recommendation—healthy (GDR-healthy)	-	A cumulative score, based upon presence food groups that make up 5 global recommendations for consumption of healthy foods	Dark green leafy vegetables Vitamin-A rich orange-coloured vegetables, roots, and tubers Other vegetables Vitamin A-rich fruits Citrus fruits Other fruits Legumes Nuts and Seeds Whole grains	0–+9
Global dietary recommendation—limit (GDR-limit)	-	A cumulative score, based upon presence food groups that make up 6 global recommendations for consumption of foods to be limited	Sodas Baked grains Other sweets Processed meat (double weighted) Unprocessed red meat Deep-fried foods Fast food Packaged Salty snacks	–9–0
Global dietary recommendation (GDR-score):	-	Sum of the GDR-limit score subtracted from the GDR-Healthy score	-	–9 to +9

*See Herforth et al. (2020a).

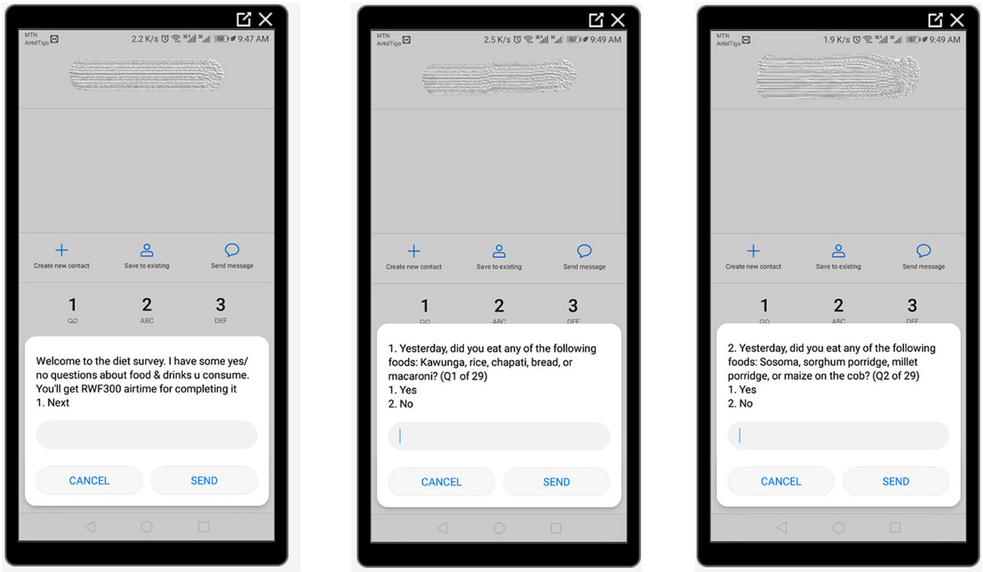

FIGURE 2 | Diet quality questionnaire presented *via*.

data collection was calculated (confidence interval of 1.96 and confidence limits of 95%). However, due to budget limitations, this was reduced to 1,800 individuals. Our experience of similar USSD-based surveys (e.g., Adewopo et al., 2021) has shown that most contacted individuals will not perform the survey, with response rates around 20% (Solano-Hermosilla et al., 2020). To address this and secure a representative sample, per data collection moment, 10,000 individuals were contacted in each weekly data collection moment.

To ensure sex-balanced responses, a 50-50 split in responses was programmed. This meant that once 50% of the required respondents were received to be male or female, no further submissions from that sex group could be submitted. To ensure spatially distributed and representative responses, we implemented a population-based weighting of respondents. To do this, the DQ-Q was sent proportionally to individuals in each district, based upon the population of that district, relative to the national. Once the required number of responses from each district was submitted, no further submissions from that district were accepted.

The contacted cohort of 10,000 individuals was randomly selected per week, considering the following requirements: (i) respondents provided consent to be contacted; and (ii) respondents provided information on their sex and district when consenting to be contacted by the 845 service. Although there is the possibility that potential respondents may be shortlisted multiple times for the sample cohort, we do not track the same individuals intentionally. In this pilot, we are only looking to gain insights on diet quality metrics at an administrative aggregate level. Future iterations could easily track diet quality for the same individuals across time.

Submissions

The sample cohort of potential respondents were sent a preliminary information message *via* Short Message Service (SMS). This message informs the cohort to respond if they would like to perform the DQ-Q survey. Participants then have 7 days to respond to the questionnaire, with data collection opening on Monday morning and closing on Sunday evening. Reminder messages are sent daily to those participants who respond positively to the initial message, but do not complete the survey. Respondents can drop-in and out of the survey with their responses saved. Before submitting, respondents are required to provide their sector (sub district administrative unit) and their economic group. The Rwandan government implements a social stratification of households, classifying across five categories (A-E), based upon income and asset ownership (**Table 2**).

A submission was only finalised when respondents answered all questions. Once the required number of respondents was achieved in each location and for each sex, submission of the questionnaire was no longer available. However, because USSD allows concurrent submissions, there is the possibility of exceeding the required sample—usually between 1 and 10 as the system will only close for new respondents, respondents already in the process of responding can continue till the end.

Data collection started on the 9th of August 2021 (Week 1), with results for the first 8 weeks (27th September 2021—Week 8) presented. Data collection continues until early February 2022.

Micro-Reward Incentives

To encourage engagement, we provided meagre, yet non-promissory, reward incentives in the form of airtime. Each respondent who fully completed the questionnaire received

TABLE 2 | Description of economic categories defined by the Rwandan government.

Economic category	Household income (USD/month)	Land owned (hectares)
A	>600	>10 rural or >1 urban
B	65–600	1–10 rural or 0.03–1 urban
C	45–65	0.5–1 rural 0.01–0.03 urban
D	<45	<0.5 rural <0.01 urban
E	No income due to age, disability, or disease	No assets

250RWF (∼0.25 USD) of airtime, sent *via* a digital and traceable payment platform. This incentive was sent *en masse* to all completed respondents on the Monday following their submission.

Data Processing and Analysis

An automated process of data curation, metric calculation, and visualisation was implemented using R (R Core Team, 2021).

Ethical and Institutional Approval

Approval for the data collection was received from the Rwanda National Ethics Committee (740/RNEC/2021) and the National Institute of Statistics of Rwanda (2058/2021/10/NISR), based on extensive review of the substance and merits of the innovation to support initiatives and policy-action.

RESULTS

Data Flows

The data collection system went live in early August 2021, with 8 weekly data collection moments so far completed. Within this period, 80,000 Rwandans were contacted, with 12,821 unique interactions recorded with the system (**Figure 3A**). The interaction rate fluctuated between 17 and 18%, excluding the early weeks of the project (**Figure 3B**). The completion rate, where interactions are submitted to the system, fluctuated between 60 and 90% (**Figure 3D**) with 9,726 submissions collected (**Figure 3C**). Completion of the survey took around 13 min (**Figure 3E**), with the most active day for submissions being the day of the first SMS message sent per data collection moment- Monday (**Figure 3F**). Subsequent daily reminders to respondents yielded diminishing returns of responses.

Through preliminary data curation and analyses, we noted that a significant minority of respondents were trying to "game" the system, where individuals answer yes to all questions. We found that around 100 individuals each week were reporting in this manner. Although individuals may eat food items from 26 food groups, we assumed this unlikely and that these respondents were responding yes to complete the survey rapidly and receive the payment. To address this, we added a warning at the start of the survey announcing abnormal responses would not be accepted.

Respondent Distribution

Basic respondent information is provided in **Table 3**. The collected data shows a slight female bias, females (50.22%) and males (49.78%). The distribution of respondents' ages was found to be skewed towards younger individuals, with ∼70% (6,773) under the age of 24, with only 6% (581) over 44. The economic grouping of respondents shows a normal distribution, with 85% of respondents coming from the middle-income groupings. The poorest economic grouping only represents 3% of responses.

Common Foods Items

In **Figure 4**, the presence of food groups in respondents' diets are displayed. The results suggest a core set of food groups: other vegetables (e.g., tomato, cabbage, and aubergine), tubers and starchy vegetables (e.g., potato, cassava, and plantain), dark green leafy vegetables (e.g., green amaranth, cassava leaves, and pumpkin leaves), other fruits (e.g., banana, watermelon, and pineapple), and legumes (e.g., beans and peas). These ubiquitous food groups were consumed by at least 70% of respondents. Invariably, these groups co-occurred in respondents' diets too (**Supplementary Figure S1**).

Diet Quality Metrics

To reduce the length of the Results section, we present only the results for the summary indicators from **Table 1**: Global Dietary Recommendation Score (GDR); Global Dietary Recommendation Healthy Score (GDR-Healthy); and Global Dietary Recommendation Limit Score (GDR-Limit). The results for the other 9 indicators (e.g., fruits and vegetables, dietary fibre, free sugars, and saturated) are presented in **Supplementary Figures S2–S4**. We disaggregate the results for the three focus diet quality metrics across sex, age, economic group (**Table 3**, **Figure 5**), and location (**Figure 6**). The preliminary results demonstrate that Rwandan female respondents have marginally better GDR scores (3.19), compared to their male counterparts (3.05) (**Table 3**, **Figure 5A**). Interestingly, Rwandan women were noted to have higher GDR-healthy (**Figure 5B**) and GDR-Limit (**Figure 5C**) scores. Deriving temporal trends from such a short time series is difficult, but nascent temporal fluctuations in both the healthy and limit metrics are apparent, with both peaking between Week 5-6 (mid-September 2021).

We observed that younger respondents (<24) had the lowest GDR scores of all respondents (2.84 and 3.12—**Table 3**). As respondents get older their diet quality (GDR score) improves, with some weekly exceptions (**Figure 5D**). The diet quality of the younger respondents was limited by their high consumption of food items that should be limited (2.65 and 2.51; **Table 3**), far higher than other age groups who had GDR-Limit scores of <2 (**Figure 5F**). In contrast, elder groups were observed to have consistently lower GDR-Healthy scores (**Figure 5E**), with the eldest age group having average GDR-Healthy scores of 5.06, notably lower than the youngest group (5.50; **Table 3**).

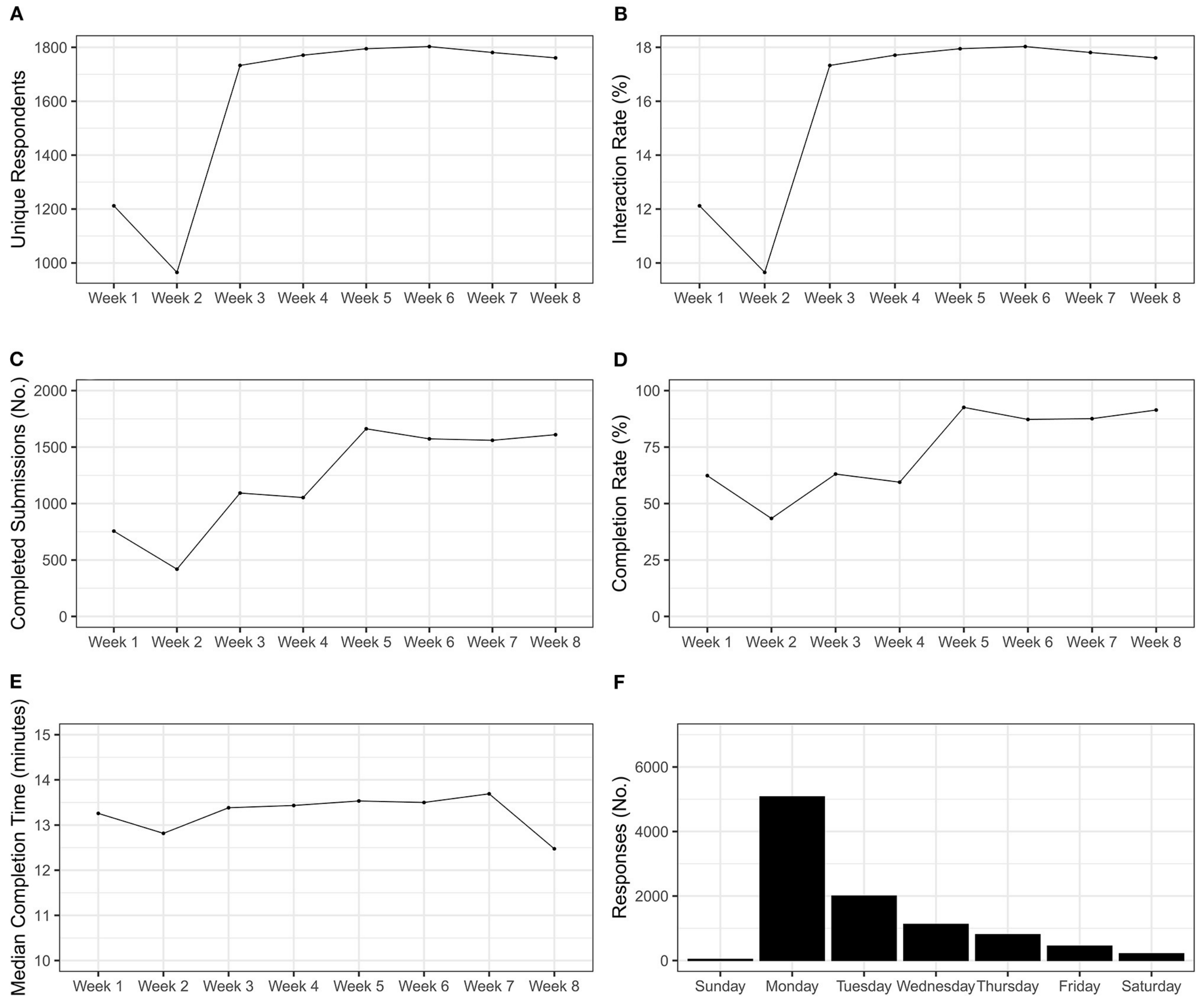

FIGURE 3 | Digital survey insights. **(A)** Total number of respondents per week of data collection. **(B)** Percentage of respondents who interacted with the survey following initial push messages. **(C)** Total number of completed submissions per week of data collection. **(D)** Percentage of respondents who completed the survey after starting an interaction. **(E)** Average time (in minutes) taken by respondents to complete the survey. **(F)** Number of unique responses per day across all data collection weeks.

The relationship between economic category and diet quality highlights that individuals in the extreme categories are those with the lowest diet quality (**Table 3**). Individuals from the wealthiest category had especially low diet quality with a GDR score of 2.92, with individuals from the poorest category only having marginally better scores of 3.04. This pattern holds largely true throughout the data collection (**Figure 5G**). These GDR scores can be explained by wealthier individuals eating moderate levels of healthier foods (**Figure 5H**), but some of the highest amounts of unhealthy foods (**Figure 5I**). Individuals with the best diet quality were found in category D, with an average GDR Score of 3.22 (**Table 3**).

In **Figure 6**, we map the mean GDR, GDR-Healthy, and GDR-Limit scores by district. These maps reflect the scores for all respondents from within each district. We found that the urban districts with the highest population densities (see **Figure 1**) are associated with the lowest GDR Score. Respondents in these locations also had the highest GDR-Healthy and GDR-Limit scores. Rural, less densely populated areas were found to have notably lower healthy and limit scores, for example in northeastern and southern Rwanda.

DISCUSSION

Understanding diet quality across socio-economic groupings, location, and time is critical to guide policy decisions and nutrition interventions to support health outcomes. Popkin et al. (2020) suggest that this requires innovative systems and tools to generate insights about diet quality across domains. We have

TABLE 3 | Summary of respondents by group.

	Respondents (No.)	Percentage	GDR- score (mean ± SD)	GDR- healthy score (mean ± SD)	GDR- limit score (mean ± SD)
Total	9,726	100			
Gender					
Female	4,844	50.22	3.19 (2.13)	5.56 (2.20)	2.37 (2.04)
Male	4,842	49.78	3.05 (2.16)	5.39 (2.21)	2.34 (2.04)
Age range					
18 or under	2,143	22.03	2.84 (2.18)	5.50 (2.18)	2.65 (2.12)
18–24	4,630	47.60	3.12 (2.14)	5.64 (2.14)	2.51 (2.02)
25–34	1,399	14.38	3.28 (2.13)	5.23 (2.30)	1.94 (1.89)
34–44	973	10.00	3.41 (2.08)	5.23 (2.33)	1.82 (1.91)
Over 44	581	5.97	3.20 (2.13)	5.06 (2.25)	1.86 (1.99)
Economic category*					
A	1,113	11.44	2.92 (2.16)	5.35 (2.36)	2.43 (2.12)
B	2,239	23.02	3.10 (2.23)	5.69 (2.15)	2.59 (2.06)
C	4,297	44.18	3.14 (2.15)	5.61 (2.12)	2.47 (2.04)
D	1,787	18.37	3.22 (2.05)	5.01 (2.28)	1.79 (1.87)
E	290	2.98	3.04 (1.89)	5.04 (2.34)	2.00 (1.99)

Mean results for the Global Dietary Recommendation Score, Global Dietary Recommendation- Healthy Score, and Global Dietary Recommendation- Limit Score are also included per group, with standard deviations provided.
See* ***Table 2**.*

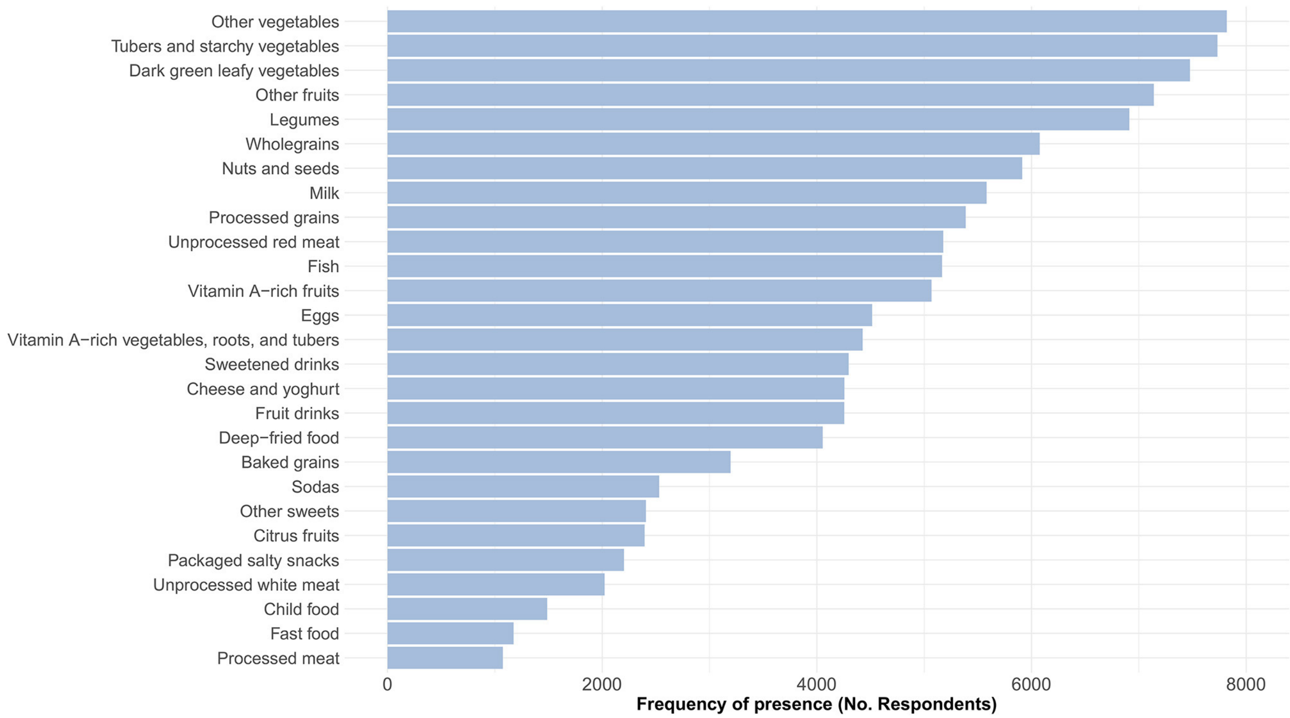

FIGURE 4 | Frequency of presence of food groups in Rwandan respondents' diets. Food groups are derived from the Diet Quality Questionnaire.

developed and tested a data collection system that crowdsources diet quality information from across Rwanda. Leveraging digital tools, we have directly engaged across socio-economic strata, age groups, and location for equitable representation of Rwandan diet quality realities in near real-time. The tool and approach reinforce the utility and benefits of digital data collection and crowd engagement and could be easily adapted to other national contexts.

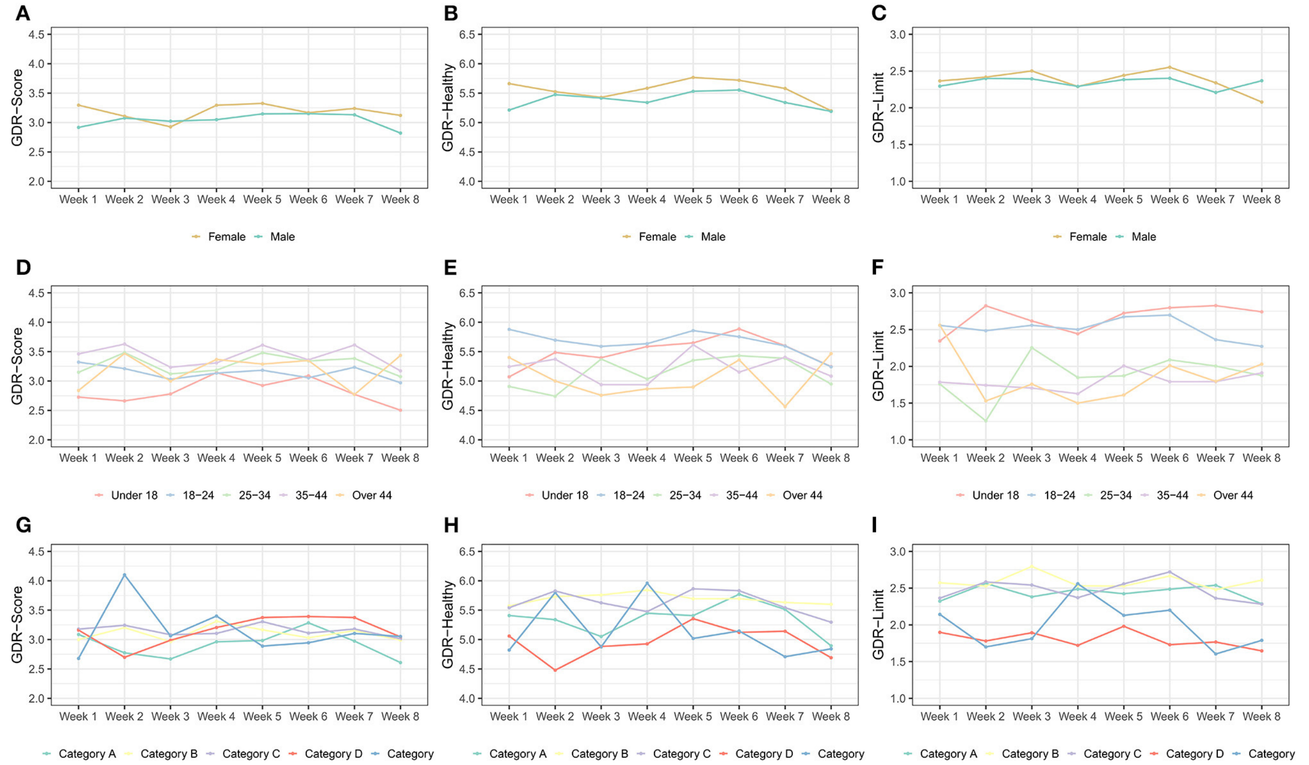

FIGURE 5 | Temporal patterns of diet quality across sex, age, and economic groups. **(A)** GDR Score across sexes. **(B)** GDR-Healthy Score across sexes. **(C)** GDR-Limit Score across sexes. **(D)** GDR Score across age groups. **(E)** GDR-Healthy Score across age groups. **(F)** GDR-Limit Score across age groups. **(G)** GDR Score across economic categories. **(H)** GDR-Healthy Score across economic categories. **(I)** GDR-Limit Score across economic categories.

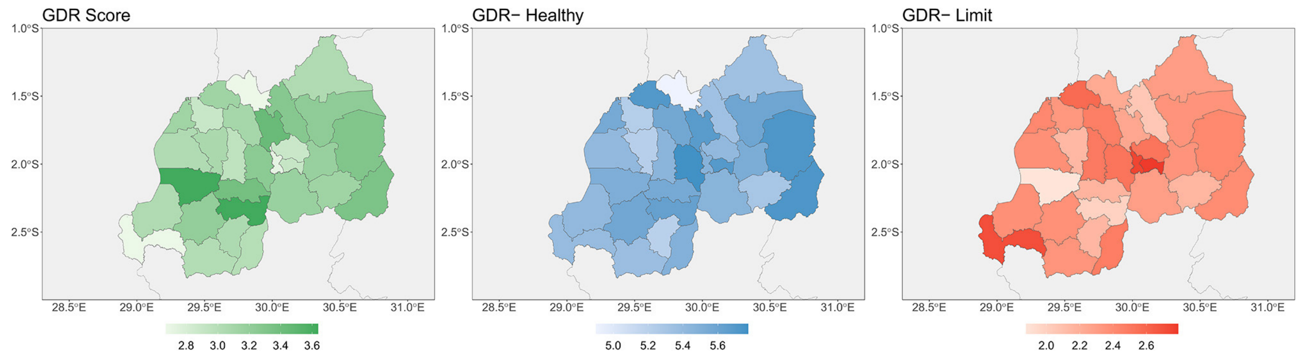

FIGURE 6 | Geographic distribution of the diet quality metrics.

Provisional Learning From a Crowd-Based Data Collection System

Developing this novel data collection system reinforced both the benefits of digital data collection and, consistent with other researchers, its shortfalls (e.g., Trucano, 2014; Lamanna et al., 2019). One of the greatest benefits is that digital data collection can be highly cost-efficient (Zeug et al., 2017; Adewopo et al., 2021). Preliminary estimates place our cost per respondent at around $1, with this cost largely associated with the system set-up and maintenance, with the incentive payment making up a small portion of the cost. There remains considerable scope for further cost-efficiencies as the system matures and automates, reducing the cost of each data collection moment. In contrast, an identical, but in-person application of the survey in Rwanda had a per respondent cost of $33 (staff costs, travel, car rental, and fuel). Further, the digital survey was more efficient in terms of time per response, taking around 15 min, compared to almost an hour for in-person application.

As noted in the results, we identified a small (<100/week), but not insignificant, cohort of respondents who were suspected of taking advantage of the simplicity of the system to receive the incentive by providing a convenient response of "yes" to all questions. These respondents were excluded from further consideration in the data analyses. Similar instances of suspected gaming/hacking have been reported in other crowd-based data collection efforts (Solano-Hermosilla et al., 2020). To mitigate this challenge, warning messages were sent to respondents whose submissions were flagged as suspicious. Based on the consideration that it is unrealistic that a person would have consumed 80% (or more) of the food classes within a 24-hr period, submissions that showed response of "yes" to more than 80% of questions were excluded. This brings one of the major concerns about digital data collection into focus, the trustworthiness of the data. This is a subject of ongoing discourse in recent literature (Adewopo et al., 2021). Crowdsourced data can be susceptible to errors and bias due to absence of direct control or verification of each datapoint at the time and location of submission. This may be linked to various factors including the clarity of questions or data required and personality/profile of the respondents (including candour, educational level, perception, age, and mental retention/recall). Yet, the advantages of crowdsourced data systems usually outweigh these limitations, especially considering the volume of data that are often generated, with little or no direct contact with respondents. According to Solano-Hermosilla et al. (2020), the robustness of acquired data provides strong leverage for high-precision data filtering which allows noisy errors to be filtered out prior to detailed data analysis, and to generate meaningful insights.

We also experienced lower response rates (18%), compared to previous similar data collection data where the response rate (or crowd engagement) hovered around 20% (Solano-Hermosilla et al., 2020). In this initial set-up and deployment, it is uncertain if the initial messaging to respondents could have significantly influenced the response rate or participation. One way to address this in future iterations would be to implement A/B testing, where sub-groups are provided different information (e.g., opening messages) or offered different incentives for completion. Previous examples of this testing mechanism have yielded results and improved the targeting and efficiencies of projects (e.g., Katmada et al., 2011; Arriagada et al., 2021).

Another issue is the representativeness of the data collected. Although sex and location-based weighting was applied to the sampling, our results are largely skewed towards younger, middle-income respondents. Although Rwandan society (and the 845# dataset) is dominated by individuals under the age of 40 (National Institute of Statistics Rwanda, 2021), our respondents appear to be an extreme reflection of the younger population in Rwanda. The demographics of Rwandan society are not truly reflected in our results, where more than 84% of respondents are below 34. The age-based skew is somewhat consistent with other similar studies (e.g., Dabalen et al., 2016), where older respondents were noted to be less likely to respond. We also found that respondents were normally distributed across the 5 economic classes, consistent with the findings of Dabalen et al. (2016).

We view these early problems as a learning curve and provide an exercise in improving the user experience and functionality of the system, rather than being a reflection of any fundamental flaws in crowd-based data collection. This learning can be used to develop and improve protocols for citizen-led, digital tool-based approaches for data collection in Rwanda and beyond.

Diet Quality in Rwanda

From the initial data collection a number of diet quality patterns were observed. The dietary composition of a "traditional" Rwandan diet emerged, with the virtual ubiquity (>70% of responses) of tubers and starchy vegetables, leafy vegetables, fruits, legumes, and wholegrains in respondents' diets. The consumption of these food items is independent of gender, location, age, or economic grouping, suggesting an almost exclusively healthy core to Rwandan diets. These findings are consistent with the findings of the Global Nutrition Report Independent Expert Group (2020), which found relatively high consumption of fruits and legumes in Rwandan diets. In contrast, we found far lower prevalence of processed meat, fatty foods, and higher prevalence of vegetables (Global Nutrition Report Independent Expert Group, 2020). We also note that dairy (excluding milk) products, processed meats, and fast foods were the least consumed food items. However, their consumption was observed to be high in younger, wealthier individuals, conforming with findings in other LMICs (e.g., Li et al., 2020).

In general, Rwandan female respondents consumed better quality diets than male respondents. Female respondents were also observed to consume a greater diversity of both healthy and unhealthy food items, consistent with global studies (e.g., Imamura et al., 2015). However, female Rwandan respondents diverge from their counterparts in other countries, who were found to consume less unhealthy food items than men (Imamura et al., 2015; Abassi et al., 2019; Darling et al., 2020).

We also note that diet quality improved with age, aligning with both Imamura et al. (2015) and Andrade et al. (2016). Respondents under the age of 24 were observed to have the lowest quality diets. Poor diet quality is widespread in adolescents (Akseer et al., 2017), driving alarming levels of obesity (WHO, 2021), and micronutrient deficiencies (Akseer et al., 2017). The impacts of low diet quality in early life have been well-documented to have considerable health, mental well-being, and life opportunity implications (e.g., Florence et al., 2008; UNICEF, 2019).

The curvilinear relationship of economic status and diet quality demonstrates that both economic extremes suffer from poor diet quality. These results reflect observed global dietary and nutritional transitions, where individuals become wealthier, shifts towards consumption of processed, sweetened, and salted food stuffs are noted (Popkin, 2015). In a recent global study, Popkin et al. (2020) observed that higher-wealth individuals have seen the greatest increases in obesity and overweight individuals in sub-Saharan Africa. These economic trends have been found to co-occur with geographical transitions, where urban populations move away from traditional diets (Steyn and McHiza, 2014).

Although we found that, generally, urban respondents consume higher amounts of healthy foods, their overall diet quality tends to be worse than rural respondents.

These early results reflect the dietary realities and point towards an underlying transition that may already be underway in Rwanda, evidenced by increased prevalence of childhood and adolescent obesity (Global Nutrition Report Independent Expert Group, 2020; Kinyonki et al., 2020). With Rwanda having one the fastest growing global economies (pre-COVID) (World Bank, 2021b), the country may be primed for continued reductions in diet quality, continued malnutrition issues, and increased prevalence of associated non-communicable diseases (GBD 2016 Risk Factors Collaborators, 2017; Marivoet et al., 2020). However, Rwanda has repeatedly demonstrated itself as being an enabling environment for pioneering data-driven decision-making to support healthy diets (Wagenaar et al., 2017). Implementation of long-term tracking of diet quality metrics, as outlined here, could help flag populations and locations with low diet quality and potential malnutrition implications to guide the allocation of resources for effective intervention. The information generated from this novel data collection system feed a dashboard (https://www.dietqualitymap.org) providing in [near] real time information on diet quality across Rwanda.

Next Steps

As data collection continues, we endeavour to identify mechanisms for improving efficiencies in the system. Envisioned content improvements include the deployment of a feedback system, where respondents become the recipients of tailored feedback to improve diet quality. This would represent a notable innovation in this field allowing respondents to be both data suppliers and information recipients. A powerful outcome of such improvements would be quantifying the extent to which dietary-based messaging can improve diet quality.

The use of the Diet Quality Questionnaire provided a validated, easy to use, and agile survey. Beyond this, its application also allows for comparison with results from a wider body of research, with the DQ-Q currently deployed across ~50 countries (Herforth et al., 2020b). Our implementation of the DQ-Q represents its first high frequency application and offers an invaluable opportunity to position our highly resolved (spatially and temporally) data for Rwanda, whilst simultaneously providing a learning opportunity for other researchers/ organisations wishing to implement similar high frequency data collection.

We will also look to contextualise our results with those of other high frequency data collections, like that of the World Food Programme's *World Hunger Map* [World Food Programme (WFP), 2021]. The aim of which being to identify if any linkages between patterns of diet quality and malnutrition can be identified for Rwanda. Our work will further explore how socio-economic, geographic, and market factors contribute to diet quality.

We will also investigate if any relationship between diet quality and weather conditions can be observed from high frequency weather data (Funk et al., 2015; Verdin et al., 2020). We hope to identify potential weather-based shocks and ascertain how, if at all, these affect regional and national diet quality.

Finally, we demonstrate how an agile and adaptable data collection system can be deployed for high frequency diet quality data collection, at minimal cost. We believe the developed system could easily be adapted to the dietary and technological contexts of other countries and are investigating potential opportunities to scale activities into neighbouring countries in Central-East Africa.

CONCLUSIONS

Innovation for food system development transcends the optimisation of on-farm production or prevention of post-harvest losses. It must also focus on consumers. Understanding and tracking diet quality is critical to guide decisions and support health outcomes. We have developed and tested an innovative system that leverages digital tools to directly crowdsource diet quality information from across Rwandan society. In collecting dietary information from 9,726 Rwandans in 8 weeks, we demonstrate the benefits of crowd-based systems deployed on digital tools for high frequency data collection, at a reasonable cost. Our preliminary results conform to dietary realities in other LMICs. We observed that female, older, and middle-income Rwandans tend to have better quality diets. The outputs also point towards nascent dietary transitions across groups. These basic insights will be complemented with further data collection, more complex analytics, and contextualisation within global studies. The sustained data flow from the system set-up constitutes an indispensable, scalable, and promising step towards robust data-driven policies and interventions for food security in LMICs. These advances are invaluable to researchers, policy-makers, and development actors who are interested in addressing diet quality and nutrition security at the most critical location(s) and time.

DATA AVAILABILITY STATEMENT

The annonymised raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, and in agreement with data availability policies of the Government of Rwanda. A fully interactive dashboard of updated project results is available at www.dietqualitymap.org.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Rwanda National Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RM and JA: idea generation, system development, analysis, and writing. MN, RR, and MS: idea generation and writing.

AG: analysis and writing. SE: system development and implementation and writing. RK: analysis. AF: system implementation and writing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.804821/full#supplementary-material

REFERENCES

Abassi, M. M., Sassi, S., El Ati, J., Gharbia, H. B., Delpeuch, F., and Traissac, P. (2019). Gender inequalities in diet quality and their socioeconomic patterning in a nutrition transition context in the Middle East and North Africa: a cross sectional study in Tunisia. *Nutr. J.* 18:18. doi: 10.1186/s12937-019-0442-6

Adewopo, J., Solano-Hermosilla, G., Colen, L., and Micale, F. (2021). Using crowd-sourced data for real-time monitoring of food price during the COVID-19 pandemic: insights from a pilot project in northern Nigeria. *Global Food Secur*. 29:523. doi: 10.1016/j.gfs.2021.100523

Akseer, N., Al-Gashm, S., Mehta, S., Mokdad, A., and Bhutta, Z. A. (2017). Global and regional trends in the nutritional status of young people: a critical and neglected age group. *Ann. New York Acad. Sci.* 1393, 3–20. doi: 10.1111/nyas.13336

Andrade, S. C., Previdelli, Á. N., Cesar, C. L., Marchioni, D. M., and Fisberg, R. M. (2016). Trends in diet quality among adolescents, adults and older adults: a population-based study. *Prevent. Med. Rep.* 4, 391–396. doi: 10.1016/j.pmedr.2016.07.010

Arriagada, J., Munizaga, M., Schwartz, D., and Mena, C. (2021). *Nudging Contributions in Crowdsourced Public Transport Technologies*. Available online at: https://ssrn.com/abstract=3795622 or http://dx.doi.org/10.2139/ssrn.3795622 (accessed December 22, 2021).

Arsenault, J. E., and Olney, D. K. (2021). Review of the micronutrient situation in Rwanda. *Food Nutr. Bull.* 42, 133–154. doi: 10.1177/0379572120975298

Beal, T., Herforth, A., Sundberg, S., Hess, S. Y., and Neufeld, L. M. (2021). Differences in modelled estimates of global dietary intake. *Lancet.* 397, 1708–1709. doi: 10.1016/S0140-6736(21)00714-5

Dabalen, A., Etang, A., Hoogeveen, J., Mushi, E., Schipper, Y., and von Engelhardt, J. (2016). *Mobile Phone Panel Surveys in Developing Countries: A Practical Guide for Microdata Collection. Directions in Development*. Washington, DC: World Bank.

Darling, A. M., Sunguya, B., Ismail, A., Manu, A., Canavan, C., Assefa, N., et al. (2020). Gender differences in nutritional status, diet and physical activity among adolescents in eight countries in sub-Saharan Africa. *Trop. Med. Int. Healthy*. 25, 33–43. doi: 10.1111/tmi.13330

Doak, C. M., Adair, L. S., Bentley, M., Monteiro, C., and Popkin, B. M. (2005). The dual burden household and the nutrition transition paradox. *Int. J. Obes*. 29, 129–36.5. doi: 10.1038/sj.ijo.0802824

Doak, C. M., Adair, L. S., Monteiro, C., and Popkin, B. M. (2000). Overweight and underweight coexist within households in Brazil, China and Russia. *J. Nutr*. 130, 2965–2971. doi: 10.1093/jn/130.12.2965

FAO. (2018). *The State of Food Security and Nutrition in the World. Building Climate Resilience for Food Security and Nutrition*. Available online at: https://www.fao.org/3/i9553en/i9553en.pdf (accessed August 21, 2021).

Ferrara, G., Kim, J., Lin, S., Hua, J., and Seto, E. (2019). A focused review of smartphone diet-tracking apps: usability, functionality, coherence with behavior change theory, and comparative validity of nutrient intake and energy estimates. *JMIR mHealth uHealth* 7:e9232. doi: 10.2196/mhealth.9232

Florence, M. D., Asbridge, M., and Veugelers, P. J. (2008). Diet quality and academic performance. *J. School Health*. 78, 209–15 doi: 10.1111/j.1746-1561.2008.00288.x

Funk, C., Peterson, P., Landsfeld, M., Pedreros, D., Verdin, J., Shukla, S., et al. (2015). The climate hazards infrared precipitation with stations - a new environmental record for monitoring extremes. *Sci. Data*. 2:150066. doi: 10.1038/sdata.2015.66

GBD 2016 Risk Factors Collaborators. (2017). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet (London, England)* 390, 1345–1422. doi: 10.1016/S0140-6736(17)32366-8

Gibson, D. G., Pereira, A., Farrenkopf, B. A., Labrique, A. B., Pariyo, G. W., and Hyder, A. A. (2017). Mobile phone surveys for collecting population-level estimates in low- and middle-income countries: a literature review. *J. Med. Int. Res*. 19:e139. doi: 10.2196/jmir.7428

Global Nutrition Report Independent Expert Group (2018). *Global Nutrition Report: Shining a Light to Spur Action on Nutrition*. Available online at: https://globalnutritionreport.org/reports/global-nutrition-report-2018 (accessed May 5, 2020).

Global Nutrition Report Independent Expert Group (2020). *Global Nutrition Report: Action on Equity to End Malnutrition*. Available online at: https://globalnutritionreport.org/reports/2020-global-nutrition-report/ (accessed October 17, 2021).

Gómez, M. I., Barrett, C. B., Raney, T., Pinstrup-Andersen, P., Meerman, J., et al. (2013). Post-green revolution food systems and the triple burden of malnutrition. *Food Policy*. 42, 129–138. doi: 10.1016/j.foodpol.2013.06.009

Herforth, A., Beal, T., and Rzepa, A. (2020b). *Global Diet Quality Project Aims to Bridge Data Gap. Gallup Blog*. Available online at: https://news.gallup.com/opinion/gallup/321968/global-diet-quality-project-aims-bridge-data-gap.aspx (accessed October 15, 2021).

Herforth, A. W., Wiesmann, D., Martínez-Steele, E., Andrade, G., and Monteiro, C. A. (2020a). Introducing a suite of low-burden diet quality indicators that reflect healthy diet patterns at population level. *Curr. Dev. Nutr*. 4:nzaa168. doi: 10.1093/cdn/nzaa168

Imamura, F., Micha, R., Khatibzadeh, S., Fahimi, S., Shi, P., Powles, J., et al. (2015). Dietary qualityamong men and women in 187 countries in 1990 and 2010: a systematic assessment. *Lancet GlobalHealth*. 3, E-132–142. doi: 10.1016/S2214-109X(14)70381-X

Katmada, A., Satsiou, A., and Loannis, K. (2011). *Incentive Mechanism for Crowdsourcing Platforms*. Springer-Verlag Berlin Heidelberg.

Kinyonki, D. K., Ross, J. M., Lazzar-Atwood, A., et al. (2020). Mapping local patterns of childhood overweight and wasting in low- and middle-income countries between 2000-2017. *Nat. Med*.

Lamanna, C., Hachhethu, K., Chesterman, S., Singhal, G., Mwongela, B., Ng'endo, M., et al. (2019). Strengths and limitations of computer assisted telephone interviews (CATI) for nutrition data collection in rural Kenya. *PLoS ONE* 14:e0210050. doi: 10.1371/journal.pone.0210050

Li, L., Sun, N., Zhang, L., Xu, G., Liu J., Hu, J., et al. (2020). Fast food consumption among young adolescents aged 12–15 years in 54 low- and middle-income countries. *Global Health Action* 13:1. doi: 10.1080/16549716.2020.1795438

Marivoet, W., Ulimwengu, J. M., and Maty Sall, L. (2020). *Rwanda: Policy Atlas on Food and Nutrition Security*. Available online at: https://snv.org/assets/explore/download/policy_atlas_rw_2020-06-23_press.pdf (accessed November 29, 2021).

National Institute of Statistics of Rwanda and ICF (2020). *Rwanda Demographic and Health Survey 2019-20: Key Indicators Report*. Kigali, Rwanda, and Rockville, Maryland: NISR and ICF.

National Institute of Statistics Rwanda. (2021). *Sustainable Development Goals*. Available online at: https://sustainabledevelopment-rwanda.github.io/2-1-2/ (accessed October 19, 2021).

Pew Research Center. (2019). *Smartphone Ownership is Growing Rapidly Around the World, But Not Always Equally*. Available online at: https://www.pewresearch.org/global/2019/02/05/smartphone-ownership-is-growing-rapidly-around-the-world-but-not-always-equally/ (accessed October 25, 2021).

Popkin, B. M. (2015). Nutrition transition and the global diabetes epidemic. *Curr. Diab. Rep*. 15:64. doi: 10.1007/s11892-015-0631-4

Popkin, B. M., Corvalon, C., and Grummer-Strawn, L. M. (2020). Dynamics of the double burden of malnutritioin and the changing nutrition reality. *Lancet*. 395, 65–74. doi: 10.1016/S0140-6736(19)32497-3

R Core Team. (2021). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/ (accessed November 23, 2021).

Reinhardt, K., and Fanzo, J. (2014). Addressing chronic malnutrition through multi-sectoral, sustainable approaches: a review of the causes and consequences. *Front. Nutr*. 1:13. doi: 10.3389/fnut.2014.00013

Rwanda Utilities Regulatory Authority. (2019). *Statistics and Tariff Information in Telecom, Media and Postal Service as of the Fourth Quarter 2018*. Available online at: https://rura.rw/fileadmin/Documents/ICT/statistics/Telecom_Statistics_report_as_of_December_2018.pdf (accessed October 5, 2021).

Rwanda Utilities Regulatory Authority. (2020). *Active Mobile Cellular Telephone Subscriptions*. Available online at: https://rura.rw/fileadmin/Documents/ICT/statistics/Mobile_cellular__Statistics_report_as_of_January_2020.pdf (accessed October 5, 2021).

Solano-Hermosilla, G., Adewopo, J., Peter, H., Arbia, G., Nardelli, V., Barreiro-Hurle, J., et al. (2020). A Quality Approach to Food Price Crowdsourcing: The Case of Food Price Crowdsourcing Africa (FPCA) in Nigeria. Report of the European Commission Joint Research Center (EC-JRC), Luxembourg (2020). Available online at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC119273/fpca_a_quality_approach_to_real_time_prices_final_online.pdf (accessed October 25, 2021).

Steyn, N. P., and McHiza, Z. J. (2014). Obesity and the nutrition transition in Sub-Saharan Africa. *Ann. New York Acad. Sci.* 1311, 88–101. doi: 10.1111/nyas.12433

Trucano, M. (2014). *World Bank Blogs: Using Mobile Phones in Data Collection: Opportunities, Issues and Challenges*. Available online at: https://blogs.worldbank.org/edutech/using-mobile-phones-data-collection-opportunities-issues-and-challenges (accessed September 10, 2021).

Tuffrey, V., and Hall, A. (2016). Methods of nutrition surveillance in low-income countries. *Emerg. Themes Epidemiol*. 13:4. doi: 10.1186/s12982-016-0045-z

UNICEF. (2019). *The State of the World's Children*. Available onlin at: https://www.unicef.org/documents/state-of-worlds-children-2019-regional-briefs (accessed October 1, 2021).

Verdin, A., Funk, C., Peterson, P., Landsfeld, M., Tuholske, C., and Grace, K. (2020). Development and validation of the CHIRTS-daily quasi-global high-resolution daily temperature data set. *Sci. Data*. 7:303. doi: 10.1038/s41597-020-00643-7

Vorster, H. H., Kruger, A., and Margetts, B. M. (2011). The nutrition transition in Africa: can it be steered into a more positive direction? *Nutrients*. 3, 429–441. doi: 10.3390/nu3040429

Wagenaar, B. H., Hirschhorn, L. R., Henley, C., Gremu, A., Sindano, N., and Chilengi, R. (2017). The AHI PHIT Partnership Collaborative. Data-driven quality improvement in low-and middle income country health systems: lessons from seven years of implementation experience across Mozambique, Rwanda, and Zambia. *BMC Health Serv. Res*. 17:830. doi: 10.1186/s12913-017-2661-x

Wells, J. C., Sawaya, A. L., Wibeak, R., Mwangome, M., Poullas, M. S., Yajnik, C. S., et al. (2019). The double burden of malnutrition: aetiological pathways and consequences for health. *Lancet*. 395, 75–88. doi: 10.1016/S0140-6736(19)32472-9

WHO. (2021). *World Health Statistics*. Available online at: https://apps.who.int/iris/bitstream/handle/10665/342703/9789240027053-eng.pdf (accessed September 18, 2021).

World Bank (2019). *The World Bank in Rwanda*. Available online at: https://www.worldbank.org/en/country/rwanda/overview (accessed May 7, 2020).

World Bank (2021a). *PovcalNet: An Online Analysis Tool for Global Poverty Monitoring*. Available online at: http://iresearch.worldbank.org/PovcalNet/home.aspx (accessed March 17, 2021).

World Bank (2021b). *The World Bank in Rwanda*. Available online at: https://www.worldbank.org/en/country/rwanda/overview#1 (accessed October 25, 2021).

World Food Programme (WFP) (2021). *Hunger Map*. Available online at: https://hungermap.wfp.org/ (accessed October 21, 2021).

Zeug, H., Zeug, G., Bielski, C., Solano-Hermosilla, G., and M'barek, R. (2017). *Innovative Food Price Collection in Developing Countries, Focus on Crowdsourcing in Africa; EUR 28247*. Luxembourg: Publications Office of the European Union.

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The Association Between Dietary Antioxidant Micronutrients and Cardiovascular Disease in Adults in the United States: A Cross-Sectional Study

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Background: Antioxidant micronutrients represent an important therapeutic option for the treatment of oxidative stress-associated cardiovascular diseases (CVDs). However, few studies have evaluated the relationship between the levels of multiple dietary antioxidants and CVDs.

Objective: The study therefore aimed to evaluate associations between dietary antioxidants and total and specific CVDs among a nationally representative sample of adults in the US.

Design: In total, 39,757 adults (>20 years) were included in this cross-sectional study from the 2005–2018 National Health and Nutrition Examination Survey. We analyzed dietary recall of 11 antioxidant micronutrients in this population. Multivariate logistic and weighted quantile sum (WQS) regression were both applied to examine the relationships between these antioxidants, alone and in combination, with the prevalence of all CVDs and specific CVDs. The linearity of these correlations was also explored using restricted cubic spline (RCS) regression.

Results: Multivariate logistic models showed that, compared with the lowest quartile, the levels of 11 antioxidants in the highest quartile were independently associated with decreased total CVD (all $P < 0.05$). The WQS index showed that, when considered together, the 11 micronutrients were negatively correlated with total CVD ($P < 0.001$) and five specific CVDs (all $P < 0.05$), and selenium had the strongest association (weight = 0.219) with total CVD. Moreover, the RCS model demonstrated that iron, zinc and copper were all negatively and non-linearly correlated with total CVD, while the eight other micronutrients had non-significant, linear, negative relationships with total CVD (P for non-linearity >0.05). A piecewise binary logistic regression analysis showed that the inflection points in the relationships between CVD and iron, zinc and copper were 7.71, 6.61, and 0.74 mg/day, respectively.

Conclusions: Our findings suggested that high levels of combined dietary antioxidant micronutrients are associated with decreased prevalence of CVDs, and that selenium has the greatest contribution to this association.

Keywords: antioxidant micronutrients, dietary nutrient intake, cardiovascular disease, disease nutrition interaction, weight quantile sum, restricted cubic spline, US adults

HIGHLIGHTS

- We analyzed 11 dietary antioxidant micronutrients in collaboration with total cardiovascular disease (CVD) and specific CVDs in a nationally representative United States population.
- Dietary intake of 11 antioxidant micronutrients were independently associated with decreased odds of total CVD and specific CVDs.
- Weighted quantile sum (WQS) analysis showed that, when considered together, the 11 antioxidants were negatively associated with the odds of total CVD and specific CVDs; of all components, selenium was most strongly associated with total CVD.
- Iron, zinc and copper were all non-linearly, negatively correlated with total CVD; inflection points were at 7.71, 6.61, and 0.74 mg/day, respectively.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death and disability in both developed and developing countries, and has become a serious public health problem (1, 2). Numerous studies have been published on CVD risk factors in recent decades; these have established that aging, smoking, obesity, cholesterol levels, poor dietary habits, educational level, blood pressure, diabetes, and genetics, all affect risk (3). At-risk populations would benefit from the effective management of these risk factors. Strikingly, 43.9% of the US adult population is still projected to have some form of CVD by 2030 (4). Epidemiological studies have also shown that 75% of pre-mature CVDs are preventable through early intervention (5). A more comprehensive understanding of CVD etiology and its underlying mechanisms remain a priority.

Oxidative stress plays an important role in the progression of various CVDs, including atherosclerosis, heart failure, cardiac arrhythmia, and ischemia-reperfusion injury (6, 7). Increased oxidative stress can modify DNA and proteins, induce subcellular remodeling and $Ca2^{+}$-handling abnormalities, and lead to functional hypoxia and disordered metabolism; this can result in cellular inflammation and programmed cell death, necrosis, and fibrosis, which are tightly regulated by reactive oxygen species (ROS) production and intracellular defense mechanisms (8, 9). There are a number of therapeutic options available for the treatment of oxidative stress-associated CVDs. Among these, micronutrients are critical for every stage of the antioxidant response. Fundamental studies on the properties of antioxidant micronutrients have shown that the fat-soluble vitamins A (including retinol, carotene and cryptoxanthin) and E (α-tocopherol) and the water-soluble vitamin C (ascorbic acid) all have anti-inflammatory properties (10). These vitamins interact with free radicals and reactive nitrogen species (RNS), decreasing polyunsaturated fatty acid (PUFA) peroxidation and thus protecting cell membrane phospholipids and plasma lipoproteins, with beneficial effects on glutathione (GSH) status and oxidation defense capabilities (11). Similarly, increasing evidence indicates that essential metal micronutrients, such as selenium, zinc, copper, and iron, play critical roles in a wide range of physiological processes; they are integral to the enzymatic system involved in the reduction of oxygen free radicals (12). It is possible that inadequate intake of these nutrients may result in increased ROS, which have an implied role in the mechanisms and risk of CVD.

Observational studies and clinical trials have evaluated the potential role of antioxidant micronutrients and their safe dose for CVD prevention or treatment (13). Epidemiological studies of individual micronutrients reported that dietary vitamin A, carotenoids (14, 15), vitamin C (16, 17), vitamin E (18), selenium (19, 20), zinc (21), iron (22), and copper (23) were associated with lower CVD risk and reduced cardiovascular mortality. These correlations were even more pronounced in the deficiency state of subsequent complement micronutrients and the level of dietary metal micronutrients are closely related to serum concentrations. The metabolism and distribution of various trace vitamins and minerals also alter cellular ion contents, which in turn modulates the metabolic functions of macronutrients (carbohydrates, lipids, and proteins) and levels of probiotic bacteria; this affects intrinsic pathological mechanisms of CVD and cumulative drug therapeutic effects in people with this disease (22), and thus impacts long-term pharmacotherapy.

Notably, the effect of antioxidant micronutrients, alone or in combination, in the prevention or reduction of CVD is controversial. Analyses from 15 trials reporting data on 188,209 participants showed that supplementation of the antioxidant vitamins E and C and β-carotene has no effect on the incidence of major cardiovascular events, myocardial infarction, stroke, total deaths, and cardiac-related deaths (24). Other studies have even suggested that supplementation of antioxidants may increase CVD risk owing to potential peroxidation (23). There are several challenges to overcome in studies on the impact of dietary antioxidants relating to dosage, duration of intervention, and baseline micronutrient status of those receiving interventions, in particular, the interactive effects between vitamins cannot be ignored (25). There is a rapidly growing interest in the health effects of dietary exposure to combinations of dietary antioxidant micronutrients because mixed exposure better represents the mixed diets that people eat in real-world situations. Studies on micronutrient status and the effects of combined supplementation are therefore worthy of further study, but they pose unique challenges to inference (26, 27); few studies have been conducted in this domain. Statistical techniques for analyzing exposure to combined factors have been developed, including weighted quantile sum (WQS), which permits the assessment of the overall effect of combined treatments on health outcomes, and crucially, the weighted contributions of each component used to answer questions about projecting exposures to a higher dimensional space (28).

The National Health and Nutrition Examination Survey (NHANES), which collects detailed data on diet, nutritional status, and chronic disease to inform nutrition and health policy, is the cornerstone for national nutrition monitoring in the US (29). The objectives of the current study were to present an analysis using this large, cross-sectional, nationally-representative database to assess the intake status of v antioxidant micronutrients with a known role in CVD, and explore the effects of combined exposure to these antioxidants on CVD.

METHODS

Study and Population

This study used data from NHANES, a nationally-representative, cross-sectional database on civilian, non-institutionalized persons living in the United States, administered by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention. NHANES surveys are demographically based, with samples selected through a complex, multistage survey design (30). A detailed sample design is available at: https://wwwn.cdc.gov/nchs/nhanes/tutorials/module2.aspx). Specifically, we used the NHANES 2003–2018 continuous survey. The data in this survey were collected using a series of large, complex, stratified, multistage probability samples with a 4-year design, with data released in 2-year cycles. Our analysis used data on dietary recall of vitamin intake, collected from the What We Eat in America (WWEIA) component of the NHANES, which is conducted by a partnership between the US Department of Agriculture (USDA) and the US Department of Health and Human Services (DHHS) (31).

Participants provided written informed consent and all study procedures were approved by the National Center for Health Statistics Research Ethics Review Board. None of the authors of this study have ever been involved in the collection or production of the NHANES database. Participants with fewer than two valid 24-h dietary recalls, and those who were younger than 20 years of age for whom data on CVD outcomes were missing, were excluded from our analyses during the 2003–2018 period.

Measurement of Two 24-h Diet Recalls

Dietary intake data for all participants were used to estimate the types and amounts of foods and beverages (including all types of lipids volume) consumed during the 24-h period prior to a dietary recall interview (midnight to midnight), and to estimate intakes for energy, nutrients, and other food components. All NHANES participants were eligible for two 24-h dietary recall interviews; the first was collected in person in a mobile examination center and the second was collected by telephone, 3–10 days after the first 24-h recall period (30). In the dietary recall investigation in NHANES 2003–2018, two dietary interviews were conducted with all sample members.

Data on intake for 11 antioxidant related micronutrients [vitamin C (DRTVC), iron (DRTIRON); vitamin E as α-tocopherol (DRTATOC); zinc (DRTZINC); β-carotene (DRTBCAR); copper (DRTCOPP); α-carotene (DRTACAR), vitamin A (DRTVARA); retinol (DRTRET); β-cryptoxanthin (DRTCRYP); selenium (DRTSELE)] were included in our analysis. (https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/DR1IFF_J.htm) (https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/DR2IFF_J.htm#Appendix_2). The average of two 24-h dietary recall values for each antioxidant in each 2-year cycle from the 16 years of available data was analyzed. These amounts reflected only nutrients obtained from foods, beverages, and water, including tap and bottled water (32); they did not include nutrients obtained from dietary supplements, antacids, or medications.

CVD Definition

Participants aged ≥20 years old were asked: "Has a doctor or other health professional ever told you that you have X," where X was congestive heart failure (CHF), coronary heart disease (CHD), angina, heart attack, or stroke. The CVD data in the NHANES 2003–2018 survey was obtained from medical records, laboratory data, and questionnaires. In addition, the data for each CVD outcome were gathered for further analysis of their association with dietary antioxidant micronutrients.

Covariates

Potential confounding variables for the CVD outcome measures were collected. Sociodemographic characteristics included age in years (modeled continuously), sex (male or female), race/ethnicity ("non-Hispanic white," "non-Hispanic black," "Mexican American," "other Hispanic," and "other," including multi-racial), educational level (above high school, high school, and below high school), working status, and poverty status. Health-related covariates included body mass index (BMI; kg/m^2); smoking status, as defined by responses to two questions ("Have you smoked at least 100 cigarettes during your entire life?") (https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/SMQ_J.htm); and habitual drinking status, in which someone was defined as drinking if they answered "yes" to "In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?" and "In the past 12 months did you have at least 12 drinks of any kind of alcoholic beverage?" (https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/ALQ_J.htm). We also collected data on comorbidities, including diabetes mellitus and hypertension, as determined through a questionnaire administered by NHANES personnel (Subcommittee of Professional and Public Education of the American Heart Association Council) (https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/DIQ_J.htm); in addition, dietary supplement intake was recorded in another questionnaire (https://wwwn.cdc.gov/nchs/nhanes/Search/default.aspx.) (33).

Statistical Analyses

Eleven antioxidant micronutrients were analyzed for their association with CVD overall and with CHD, CHF, heart attack, stroke and angina separately. Normality of continuous variables was assessed using Kolmogorov–Smirnov-tests. Continuous variables were expressed as mean [standard deviation (SD)] and were compared using unpaired *t*-tests. Categorical or dichotomous variables were expressed as absolute value (percentage) and were compared using χ^2-tests. The correlation coefficients for all antioxidant micronutrient dietary intakes were calculated using the Pearson correlation method. The metabolites of antioxidant micronutrients were divided into quartiles, and the lowest quartile was used as a reference category. Concentrations of each of the 11 micronutrients were log-transformed to normalize their distributions for further analysis.

Multivariate logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to assess the aggregate specific CVDs prevalence associated with each of the 11 micronutrients. Three models were used, with increasing levels of adjustment for confounding variables: in model 1, data were adjusted for age and sex; model 2 was based on model 1, with additional adjustments for race, education levels and poverty; and model 3 was based on model 2, with additional adjustments for smoking, drinking, BMI, total cholesterol, dietary supplement use, diabetes, and hypertension.

Because of high correlations between dietary intake values for various vitamins, we performed WQS regression using the gWQS in R v.3.6.1 (34). These analyses were used to assess associations between the level of all antioxidant micronutrients in combination and CVD, and to evaluate these as predictors of CVD in logistic regression models. Each micronutrient was assigned a weight within the index that indicates its contribution to the overall association (35). In the gWQS function, we used deciles for exposure weighting, 1,000 bootstrap repetitions, a random seed set to 2018, and a binomial distribution for the general linear model. A constraint of this approach is that, in each case, weights are estimated by pooling effects only in the positive or the negative direction. Individual vitamin weights of ≥0.1 were considered significant contribution rate. The value of 0.1 was chosen for ease of comparison across models that included different numbers of antioxidants.

The shape of the relationship between dietary micronutrient intake and risk of CVD were explored using the restricted cubic spline (RCS) regression model with three knots (10th, 50th, and 90th) percentile of antioxidants and analysis of variance (ANOVA) was used to test for non-linearity (36). If non-linearity was detected, segmented regression was used to fit the piecewise-linear relationship between micronutrients and total CVD or specific CVDs, and to calculate the threshold inflection point using a recursive algorithm, as described previously (37). The significance of each interaction (*P* interaction) was tested using the likelihood ratio test. Significance was set to $P < 0.05$ (two-sided).

RESULTS

Study Population Characteristics According to Total CVD Status

In total, 80,312 people were initially enrolled. Ten thousand seven hundred and thirteen participants with fewer than two valid 24-h dietary recalls and 29,842 people younger than 20 years of age with missing data on CVD outcomes were excluded, leaving a total of 39,757 participants who were included in our analyses (**Figure 1**), comprising 19,279 (48.5%) male and 20,460 (47.5%) female participants, with a mean age of 49.6 ± 18.0 years old. For the purposes of our analysis, we separated these into those without CVD (n = 35,280, 47.5%) and those with CVD (n = 4,477, 56.6%). **Table 1** shows the demographic characteristics of these two groups. Participants with CVD were generally older (66.7 ± 13.0 vs. 47.5 ± 17.4), comprising a greater proportion of men (56.6 vs. 47.5%), more high-school educated individuals (25.3 vs. 23.0%), more non-Hispanic participants (55.5 vs. 42.5%), higher poverty states (23.1 vs. 21.6%), a greater proportion of smokers (61.2 vs. 43.3%), higher BMI (7.0 vs. 6.8%), greater dietary supplement use (59.1 vs. 49.9%), and greater rates of diabetes (33.0 vs. 10.1%) and hypertension (73.4 vs. 31.1%).These differences between the two groups were statistically significant (all $P < 0.05$).

Distribution of and Correlation Between Antioxidant Micronutrient Levels

Overall, **Supplementary Table S1** shows the detected concentrations and distribution of the 11 antioxidant micronutrients, with the highest levels observed for vitamin C, followed by iron, vitamin E, zinc, β-carotene, copper, α-carotene, vitamin A, retinol, β-cryptoxanthin, and lowest levels observed for selenium.

Correlation analyses showed that most of the antioxidant micronutrients were moderately correlated with the other 10 vitamins (Spearman's rank $r \geq 0.3$). An r value of ≥0.7 was found in the correlations between zinc and iron, α-carotene and β-carotene, selenium and zinc, and zinc and copper (all $P < 0.001$). An *r*-value of ≥0.5 was found between iron and copper, β-carotene and α-carotene, selenium and iron, vitamin A and β-carotene, selenium and copper, retinol and vitamin A, vitamin c and β-carotene, vitamin E and copper, copper and vitamin A, and vitamin c and β-cryptoxanthin (all $P < 0.001$), as shown in **Figure 2**.

Associations Between 11 Antioxidant Micronutrients and Total CVD

The 11 antioxidant micronutrients were divided into quartiles, and the reference category was considered to be the lowest quartile. The results from the multivariable logistic regression models adjusted for the covariates to assess the prevalence rates of total CVD associated with antioxidant micronutrients are shown in **Table 2**. Using model 1, 11 antioxidant micronutrients were found to have negative association with total CVD when comparing the second, third, and fourth quartiles with the reference quartile, respectively (all *P* for trend <0.05). Further

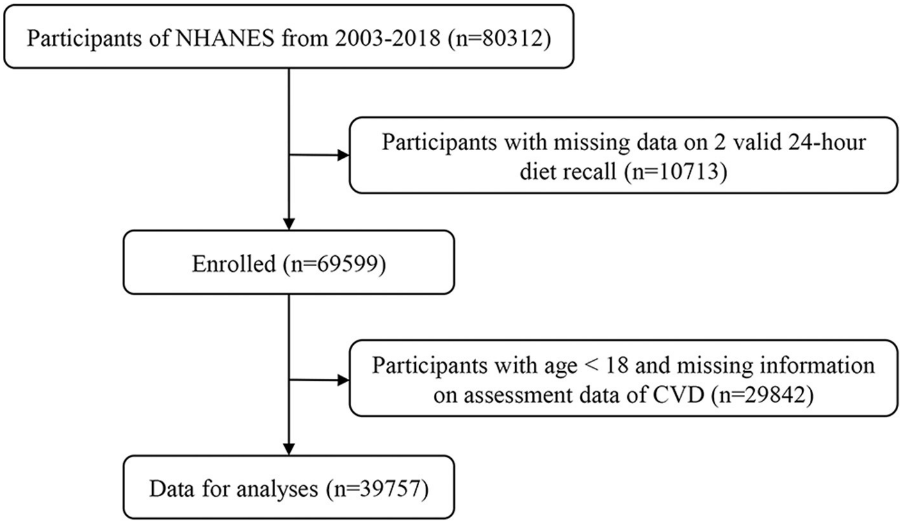

FIGURE 1 | Study flow diagram.

TABLE 1 | Sociodemographic characteristics of the study population.

Variable	Total (n = 39,757)	Non-CVD (n = 35,280)	CVD (n = 4,477)	P-value
Age, years, Mean ± SD	49.61 ± 18.04	47.46 ± 17.43	66.73 ± 12.9	<0.001
Male, N (%)	19,279 (48.49%)	16,746 (47.47%)	2,533 (56.58%)	<0.001
Education level, N (%)				<0.001
Below high school	10,033 (25.24%)	8,524 (24.16%)	1,509 (33.71%)	
High school	9,264 (23.30%)	8,131 (23.05%)	1,133 (25.31%)	
Above high school	20,460 (51.46%)	18,625 (52.79%)	1,835 (40.98%)	
Race/ethnicity, N (%)				<0.001
Mexican American	6,413 (16.13%)	5,948 (16.89%)	465 (10.39%)	
Other Hispanic	3,482 (9.66%)	3,193 (9.05%)	289 (6.45%)	
Non-Hispanic White	17,481 (43.97 %)	14,996 (42.51%)	2,485 (55.51%)	
Non-Hispanic Black	8,511 (21.41%)	7,546 (21.39%)	965 (21.55%)	
Other race	3,870 (9.73%)	3,597 (10.20%)	273 (6.10%)	
Poverty, N (%)	8,315 (20.91%)	7,280 (20.63%)	1,035 (23.12%)	0.007
Smoker, N (%)	18,021 (45.32%)	15,281 (43.31%)	2,740 (61.20%)	<0.001
Drinking, N (%)	27,962 (70.33%)	24,947 (70.71%)	3,015 (67.34%)	
Body mass index, kg/m^2, Mean ± SD	29.15 ± 6.87	29.04 ± 6.77	30.33 ± 7.29	<0.001
Total cholesterol, mmol/L, Mean ± SD	4.86 ± 1.13	5.14 ± 1.13	4.72 ± 1.07	<0.001
Dietary supplement use, N (%)	20,241 (50.91%)	17,596 (49.88%)	2,645 (59.08%)	<0.001
Diabetes, N (%)	5,039 (12.67%)	3,562 (10.10%)	1,477 (32.99%)	<0.001
Hypertension, N (%)	14,242 (35.82%)	10,956 (31.05 %)	3,286 (73.40%)	<0.001

CVD, cardiovascular disease.
Data are presented as mean (SD) or median (interquartile range), or n (%).

analysis known risk factors were used as covariates to reduce false positives induced by multiple corrections both in model 2 and 3. The second, third, and fourth quartiles of 11 antioxidant micronutrients were independently associated with the decreased prevalence of total CVD compared with the lowest reference quartile (all P for trend < 0.05).

In model 3, when comparing the fourth quartiles of each antioxidant micronutrients with the reference quartile, vitamin E (OR, 0.74; 95% CI, 0.67–0.82); retinol (OR,0.85; 95% CI, 0.77–0.94); vitamin A (OR, 0.75; 95% CI, 0.68–0.83), α-carotene (OR, 0.74; 95% CI, 0.67–0.82), β-carotene (OR, 0.75; 95% CI, 0.68–0.83), and β-cryptoxanthin (OR, 0.90; 95% CI, 0.81–0.99),

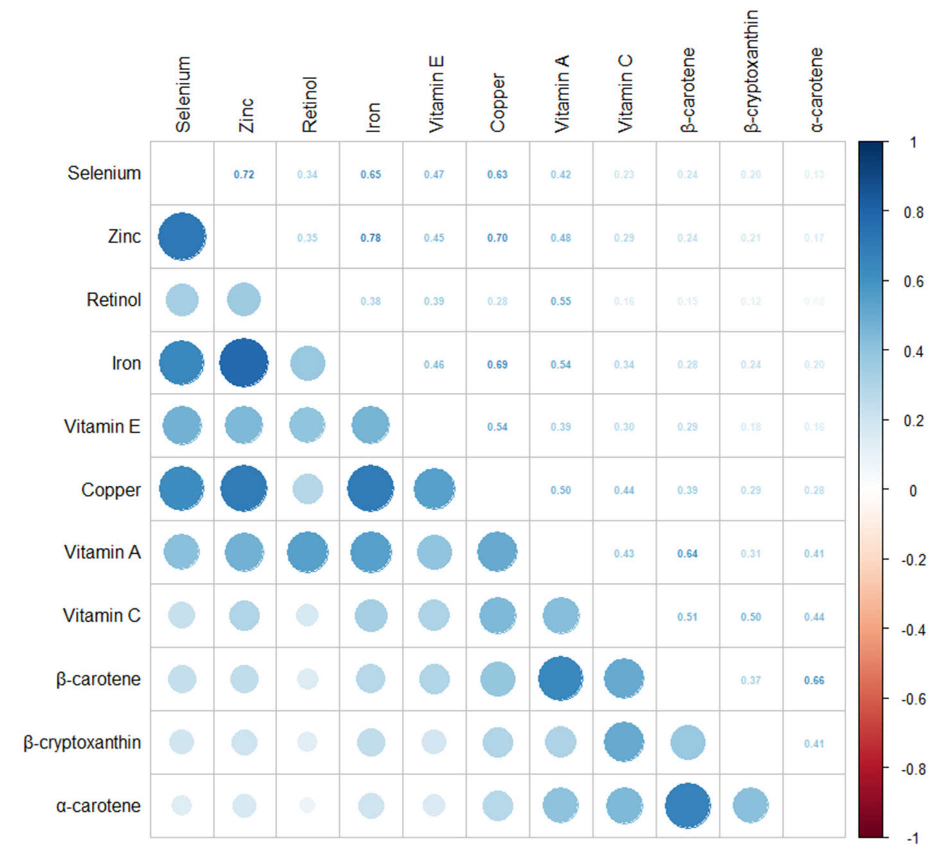

FIGURE 2 | Pairwise Pearson correlation coefficients between dietary intake of 11 antioxidant micronutrients in adults from the United States, collected from the National Health and Nutritional Examination Survey (NHANES) database 2003–2018.

vitamin C (OR, 0.81; 95% CI, 0.73–0.90), iron (OR, 0.74; 95% CI, 0.66–0.82), zinc (OR, 0.76; 95% CI, 0.68–0.85), selenium (OR, 0.67; 95% CI, 0.60–0.75) and copper (OR, 0.67; 95% CI, 0.60–0.75) had a lower odds ratio, respectively. The results showed significant protective correlation existed between 11 antioxidant micronutrients and total CVD.

WQS Regression Analysis of Negative Relationships Between the 11 Antioxidant Micronutrients in Combination and Total and Specific CVDs

The negative relationships between the combined antioxidant micronutrients and prevalence rates of total and specific CVDs were analyzed using WQS regression analysis. The combined index for the 11 antioxidant micronutrients was independently correlated with total CVD (adjusted OR, 0.79; 95% CI, 0.74–0.84; $P < 0.001$), CHF (adjusted OR, 0.82; 95% CI, 0.73–0.91; $P < 0.001$), CHD (adjusted OR, 0.87; 95% CI, 0.79–0.96; $P = 0.005$), angina (adjusted OR, 0.89; 95% CI, 0.79–0.99, $P = 0.037$), heart attack (adjusted OR, 0.86; 95% CI, 0.79–0.94, $P = 0.001$), and stroke (adjusted OR, 0.73; 95% CI, 0.66–0.80, $P < 0.001$), as shown in **Table 3**. WQS constrains exposure–outcome associations to a negative direction.

Selenium was found to have the greatest contribution to the combined effect of the micronutrients (weight = 21.60%) in the total CVD model. Selenium, copper, β-carotene, vitamin E, and iron had weights of >0.1 in the total CVD analysis; additionally, weights of >0.1 were also observed for selenium in CHF, angina and stroke; copper in angina, heart attack, and stroke; vitamin E in CHF, CHD, and heart attack; and iron in CHF and stroke. β-cryptoxanthin had the greatest contribution of all antioxidative micronutrients in stroke (weight = 23.80%); the weight of vitamin C did not exceed 0.1 in the total CVD analysis or those of specific CVDs (as shown in **Table 4**; **Supplementary Figure S1**).

Multiple Logistic Regression Analysis for Selenium, Copper, β-Carotene, Vitamin E, and Iron and Specific CVDs

The weight of selenium, copper, β-carotene, vitamin E, and iron were exceeded 0.1, which were significant contribution in CVDs, and the relationship between the 5 antioxidants and specific CVDs was further assessed using multiple logistic regression. After adjustment using model 3, compare with the lowest quartile, as the maximum weight in total CVD model the highest selenium level remained significantly negatively associated with CHF (adjusted OR, 0.70; 95% CI, 0.58–0.84; $P = 0.001$), angina

TABLE 2 | Adjusted regression coefficients with 95% confidence intervals (95% CIs) in a multiple regression analysis for total CVD model and 11 dietary antioxidant micronutrients in adults from the United States, collected from the National Health, and Nutritional Examination Survey (NHANES) database, 2003–2018.

Micronutrients	Q1	Q2	Q3	Q4	*P* for trend
	OR	OR (95% CI)	OR (95% CI)	OR (95% CI)	
Vitamin E (mg)					
Model 1	1	0.83 (0.76–0.91)	0.72 (0.65–0.79)	0.70 (0.64–0.77)	<0.001
Model 2	1	0.86 (0.79–0.94)	0.76 (0.69–0.83)	0.75 (0.68–0.82)	<0.001
Model 3	1	0.85 (0.77–0.93)	0.75 (0.68–0.83)	0.74 (0.67–0.82)	<0.001
Retinol (μg)					
Model 1	1	0.89 (0.81–0.98)	0.87 (0.80–0.96)	0.81 (0.74–0.90)	<0.001
Model 2	1	0.91 (0.83–1.00)	0.89 (0.81–0.98)	0.82 (0.74–0.90)	0.001
Model 3	1	0.93 (0.85–1.03)	0.89 (0.81–0.99)	0.85 (0.77–0.94)	0.015
Vitamin A (μg)					
Model 1	1	0.87 (0.79–0.95)	0.80 (0.72–0.88)	0.68 (0.62–0.75)	<0.001
Model 2	1	0.89 (0.81–0.98)	0.83 (0.75–0.91)	0.71 (0.64–0.78)	<0.001
Model 3	1	0.89 (0.81–0.99)	0.85 (0.76–0.94)	0.75 (0.68–0.83)	<0.001
α-carotene (μg)					
Model 1	1	0.77 (0.69–0.84)	0.76 (0.69–0.83)	0.65 (0.59–0.72)	<0.001
Model 2	1	0.81 (0.73–0.89)	0.82 (0.75–0.91)	0.72 (0.65–0.80)	<0.001
Model 3	1	0.81 (0.73–0.89)	0.81 (0.73–0.89)	0.74 (0.67–0.82)	<0.001
β-carotene (μg)					
Model 1	1	0.83 (0.75–0.91)	0.72 (0.65–0.79)	0.65 (0.59–0.71)	<0.001
Model 2	1	0.88 (0.80–0.97)	0.78 (0.71–0.86)	0.71 (0.64–0.78)	<0.001
Model 3	1	0.86 (0.78–0.95)	0.77 (0.70–0.85)	0.75 (0.68–0.83)	<0.001
β-cryptoxanthin (μg)					
Model 1	1	0.88 (0.80–0.96)	0.79 (0.72–0.87)	0.77 (0.70–0.85)	<0.001
Model 2	1	0.91 (0.83–1.01)	0.84 (0.76–0.92)	0.83 (0.76–0.92)	<0.001
Model 3	1	0.92 (0.83–1.01)	0.85 (0.77–0.94)	0.90 (0.81–0.99)	0.012
Vitamin C (mg)					
Model 1	1	0.79 (0.72–0.87)	0.70 (0.63–0.76)	0.66 (0.60–0.73)	<0.001
Model 2	1	0.84 (0.76–0.92)	0.75 (0.68–0.82)	0.72 (0.66–0.80)	<0.001
Model 3	1	0.84 (0.76–0.93)	0.78 (0.71–0.86)	0.81 (0.73–0.90)	<0.001
Iron (mg)					
Model 1	1	0.82 (0.75–0.90)	0.75 (0.68–0.82)	0.69 (0.63–0.77)	<0.001
Model 2	1	0.85 (0.77–0.93)	0.78 (0.71–0.86)	0.73 (0.66–0.80)	<0.001
Model 3	1	0.86 (0.78–0.95)	0.79 (0.72–0.87)	0.74 (0.66–0.82)	<0.001
Zinc (mg)					
Model 1	1	0.83 (0.75–0.90)	0.79 (0.72–0.87)	0.73 (0.66–0.81)	<0.001
Model 2	1	0.86 (0.78–0.94)	0.83 (0.75–0.91)	0.76 (0.69–0.85)	<0.001
Model 3	1	0.85 (0.77–0.93)	0.85 (0.77–0.93)	0.76 (0.68–0.85)	<0.001
Selenium (μg)					
Model 1	1	0.84 (0.77–0.91)	0.77 (0.70–0.84)	0.65 (0.59–0.72)	<0.001
Model 2	1	0.86 (0.79–0.94)	0.80 (0.73–0.88)	0.70 (0.63–0.78)	<0.001
Model 3	1	0.84 (0.76–0.92)	0.77 (0.70–0.85)	0.67 (0.60–0.75)	<0.001
Copper (mg)					
Model 1	1	0.77 (0.71–0.85)	0.72 (0.65–0.79)	0.57 (0.52–0.63)	<0.001
Model 2	1	0.81 (0.74–0.89)	0.78 (0.71–0.85)	0.63 (0.57–0.70)	<0.001
Model 3	1	0.82 (0.74–0.90)	0.79 (0.72–0.87)	0.67 (0.60–0.75)	<0.001

CVD, cardiovascular disease; OR, Odd ratio; CI, confidence interval; O, quartile.

Multivariable logistic regression was conducted, and ORs were calculated while comparing the second, third, and fourth quartiles of each chemical with reference to the first exposure quartile.

Model 1 was adjusted as age and sex.

Model 2 was adjusted as model 1 plus race, education levels and poverty.

Model 3 was adjusted as model 2 plus smoking, drinking, BMI, total cholesterol, dietary supplement use, diabetes and hypertension.

TABLE 3 | WQS regression model to assess the protective association of the mixture of 11 antioxidant micronutrients with individual CVDs and total CVD risk in adults from (NHANES) database, 2003–2018.

Subgroup	OR	95% CI	*P*
CVD	0.79	(0.74–0.84)	<0.001
Congestive heart failure	0.82	(0.73–0.91)	<0.001
Coronary heart disease	0.87	(0.79–0.96)	0.005
Angina	0.89	(0.79–0.99)	0.037
Heart attack	0.86	(0.79–0.94)	0.001
Stroke	0.73	(0.66–0.80)	<0.001

CVD, cardiovascular disease; WQS, weighted quantile sum; OR, odds ratio; CI, credibility interval.
WQS regression model was adjusted as age, sex, race, education levels, poverty, smoking, drinking, BMI, total cholesterol, dietary supplement use, diabetes and hypertension.

(adjusted OR, 0.70; 95% CI, 0.57–0.85; $P = 0.002$), heart attack (adjusted OR, 0.71; 95% CI, 0.61–0.84, $P < 0.001$), and stroke (adjusted OR, 0.66; 95% CI, 0.55–0.78; $P < 0.001$), respectively (**Supplementary Table S2**). In addition, copper in the highest quartile remained significant protectively associated with CHF (adjusted OR, 0.73; 95% CI, 0.61–0.87; $P = 0.002$), angina (adjusted OR, 0.74; 95% CI, 0.61–0.89; $P = 0.017$), heart attack (adjusted OR, 0.70; 95% CI, 0.58–0.84; $P = 0.001$) and stroke (adjusted OR, 0.70; 95% CI, 0.58–0.84; $P = 0.001$), respectively (**Supplementary Table S3**). In the adjusted model, a significant association between fourth quartile of β-carotene and vitamin E and decreased 5 specific CVDs prevalence, respectively (all *P* for trend <0.05) (**Supplementary Tables S4**, **S5**); Besides, iron in the second, third, and fourth quartiles decreased risk of CHF, heart attack, and stroke compared to those in the lowest (all *P* for trend <0.05) (**Supplementary Table S6**).

Dose Response Relationship Between 11 Antioxidant Micronutrients and the Prevalence Rates of Total CVD and Specific CVDs

The median intake level of dietary iron, zinc and copper were 13.2, 9.9, and 1.1 mg, respectively (**Supplementary Table S1**). RCS and multivariate logistic regression analyses were used to flexibly model and visualize the U-shaped relationships of iron, zinc and copper with the prevalence of CVD. Iron (*P* for non-linearity = 0.006), zinc (*P* for non-linearity = 0.024) and copper (*P* for non-linearity = 0.013) all had a non-linear and negative correlation with total CVD, and total CVD events increased rapidly for iron, zinc and copper below those levels (**Figure 3**). Models predicted that the concurrent decrease in risk of CVD with decreased concentrations of iron, zinc and copper flattened out at levels of 7.71, 6.61, and 0.74 mg, respectively (**Table 5**). All eight other micronutrients had linear, negative associations (all *P* for non-linearity >0.05) (**Figure 3**).

The relationships between each of the 11 microelements and specific CVDs (CHD, CHF, heart attack, angina, and stroke) had negative non-linear and linear outcomes as shown in **Supplementary Figures S2–S6**. Among these, the association of β-cryptoxanthin with CHF, and iron, zinc, and copper with heart attack (non-linearity, $P < 0.05$) were all negative and non-linear. Linear, negative associations were found between each of the 11 microelements and stroke (all *P* for non-linearity >0.05).

DISCUSSION

Our analysis of 11 dietary antioxidant micronutrients in 39,757 US adults from a prospective, nationally representative U.S. cohort demonstrated that, after adjustment for common confounding variables, all individual 11 micronutrients were independently negatively associated with total CVD. In addition, the WQS index model showed a negative correlation between all the antioxidative micronutrients in combination and total CVD, with the greatest influence on this relationship from selenium. Negative associations were also observed between selenium and several specific CVDs (CHF, CHD, angina, heart attack, stroke). Non-linearity regression indicated a U-shaped correlation between iron, zinc and copper and total CVD, with inflection points at 7.71, 6.61, and 0.74 mg/day, respectively.

Oxidative stress exceeds the buffering capacity of the antioxidant defense systems ultimately resulting in cardiovascular dysfunction (22, 38). Laboratory data shown exogenous antioxidants, as a part of a diet are able to protect tissues from ROS and reactive RNS-induced injury (3, 39), and thus protect cells and organ systems against free radical damage. For instance, Vitamins A, C, and E and carotenoids direct the neutralization of free radicals; break the chain reaction of lipid peroxidation (40), lowering total cholesterol and low-density lipoprotein and CVD risks (41); copper, zinc, iron and selenium are required for the activity of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) diminish excessive oxidative stress (42, 43). Current our partial outcomes were consistent with previous population large meta-analyses and a few randomized controlled clinical trials, which have shown that single magnesium, copper and zinc (42, 44), selenium, carotenoids (17), vitamin C (44), and vitamin E (45, 46), are all associated with reduced prevalence of CVDs, and CVD-related death.

Despite the theoretical that these antioxidants must exert beneficial effects against oxidative stress, other prospective population trials have yielded inconsistent results on their ability to prevent CVD (47). Dosage was one of the main reasons proposed for this inconsistency. Intake of β -carotene at 15–50 mg/day had no beneficial effects on CVD outcomes in adults in a meta-analysis consisting of 15 clinical trials (40, 48). It's also notable that immoderate removal of ROS or RNS and their derived products by antioxidant supplementation may upset cell signaling pathways and might be increase the risk of chronic disease (49). Research on the effect of excess antioxidant supplementation on CVDs reported that selenium in the diet at >400 μg/day induced selenosis and heart hazards (50), and 4 years of supplementation with 20–30 mg/day β-carotene was associated with increased risk of CVD (51). Moreover, an upper limit for vitamin A (retinol) at 3,000

TABLE 4 | WQS regression analysis of 11 antioxidant micronutrients weights of total CVD and specific CVDs.

	CVD (%)	Congestive heart failure (%)	Coronary heart disease (%)	Angina (%)	Heart attack (%)	Stroke (%)
Selenium	21.60	24.80	2.80	33.00	4.64	14.10
Copper	17.10	9.07	2.48	11.40	27.70	25.00
β-carotene	16.30	19.10	7.86	8.14	5.10	9.47
Vitamin E	11.90	11.20	33.00	6.04	10.90	3.82
Rion	10.60	22.50	8.09	1.860	3.01	12.20
Vitamin C	8.62	0.14	5.50	0.29	2.53	4.25
Vitamin A	4.74	4.43	11.90	17.50	18.20	0.54
α-carotene	4.49	6.60	13.60	12.80	2.61	6.78
Retinol	2.24	0.61	2.87	6.33	18.80	0.04
Zinc	1.46	0.96	10.40	15.70	5.83	0.03
β-cryptoxanthin	0.94	0.51	1.49	00.11	0.71	23.80

CVD, cardiovascular disease; WQS, weighted quantile sum.

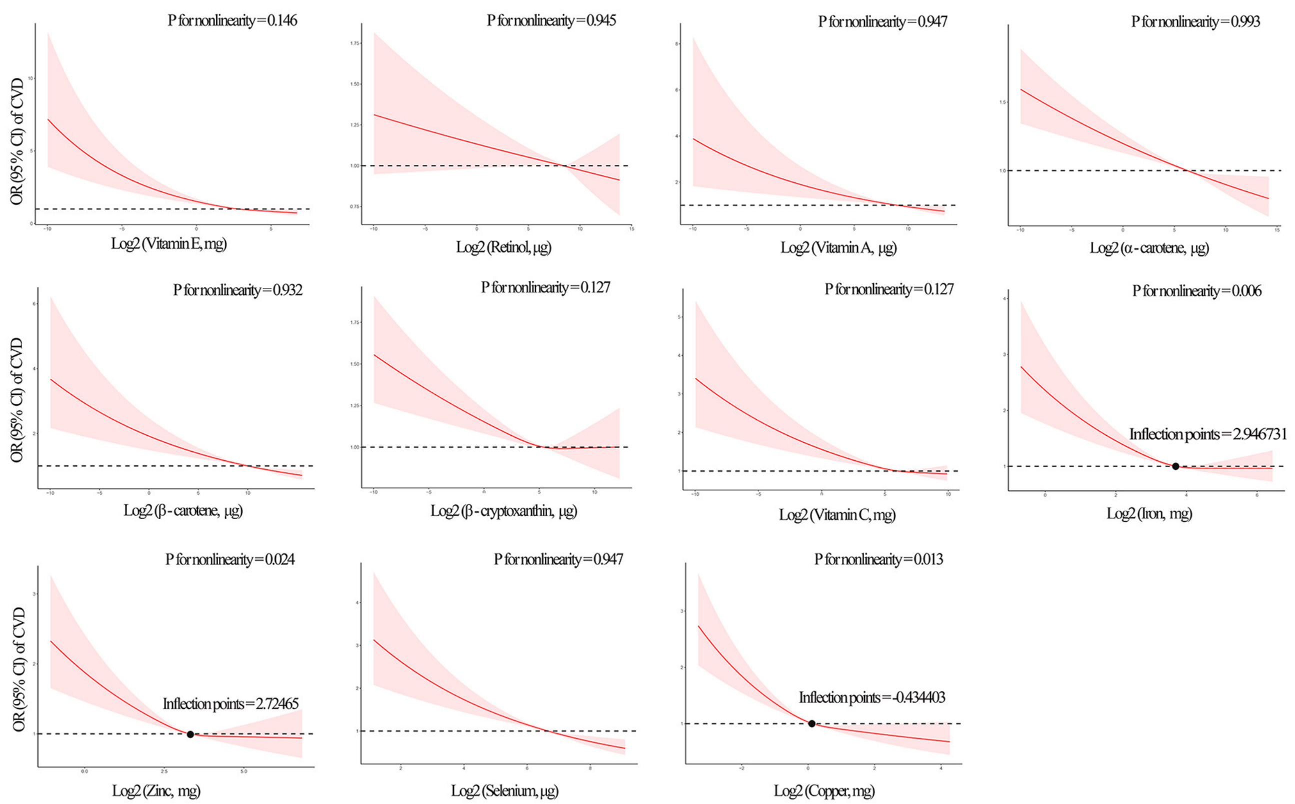

FIGURE 3 | Restricted cubic spline (RCS) analysis with a multivariate -adjusted association associations between dietary 11 antioxidant micronutrients and the prevalence of total CVD. Eleven specific antioxidant micronutrients index are modeled as restricted cubic splines with knots at the 10th, 50th, and 90th percentiles shown the non-linear association. Non-linearly related inflection points of iron, zinc and copper were annotated. The solid line is the adjusted HR; 10th percentile is used as the reference (HR = 1). The shaded area is the 95% CI of the HR. Iron, zinc and copper shown non-linearity association with total CVD model (*P* for non-linearity < 0.05).

μg/day in adults is extrapolated from a small number of case reports (15, 52).

It is difficult to obtain such high levels of trace elements from conventional foods; thus, micronutrients obtained in the diet rather than through supplements could be considered safer (45). Previous studies highlighted those whole grains, nuts, seeds, fruits and vegetables are rich in essential antioxidative micronutrients, which as role in the primary prevention of patients with CVD (9), and those who are at risk (11). Higher vegetable and nut intakes have been associated with a lower

TABLE 5 | Threshold effect analysis of iron, zinc and copper on the prevalence of total CVD risk using piecewise binary logistic regression models.

	Inflection point	group	Mean difference (95% CI)	*P*-value	*P* for log likelihood ratio test
Iron (mg)	7.71	≤7.71	0.83 (0.71–0.97)	0.018	0.221
		>7.71	0.90 (0.84–0.97)	0.007	
Zinc (mg)	6.61	≤6.61	0.75 (0.66–0.86)	<0.001	0.019
		>6.61	0.90 (0.83–0.98)	0.012	
Copper (mg)	0.74	≤0.74	0.71 (0.61–0.83)	<0.001	0.025
		>0.74	0.86 (0.80–0.94)	<0.001	

OR, Odd ratio; CI, confidence interval.
Iron, zinc and copper were log 2 transformed for fitting the piecewise binary logistic regression model.
Analyses was adjusted for age, sex, race, education levels, poverty, smoking, drinking, BMI, total cholesterol, dietary supplement use, diabetes and hypertension.

risk of stroke: an increase of 1 serving per day of green leafy vegetables intake yielded a relative risk (RR) of 0.79 (95% CI, 0.62–0.99) (42, 53); similar effects were demonstrated for myocardial infarction (0.5 servings per week vs. once a week, RR = 0.49) (39, 54). To date, antioxidants with safer dietary profile [vitamin A at 3,000 μg/day or 7,500 μg/week; α-carotenoids at up to 20 mg/day for lutein and 75 mg/day for lycopene; β-carotene at 2–4 mg/day (14, 15, 48); selenium at 55 μg/day (19) or 200 μg/day for 12 weeks (20)]; vitamin C at 500–700 mg/day (13, 14) have been confirmed, and reductions in CVDs and markers of cardiometabolic risk in adults have been observed with their use. In our study, the antioxidant micronutrients came from food rather than supplementation. **Supplementary Table S1** shown the daily 95th percentile of antioxidants (vitamin E, 21.8 mg; retinol, 1,201.0 μg; vitamin A, 1,414.0 μg; α-carotene, 1,785.1 μg; β-carotene, 7,816.1 μg; β-cryptoxanthin, 397.5 μg; vitamin C, 233.9 mg; iron, 28.7 mg; zinc, 22 mg; selenium, 207.8 μg; and copper, 2.3 mg) within safe limits had a cardiovascular protective effect after adjustment for confounding factors in our multiple logistic regression. Our non-linear regression indicated that intakes of iron, zinc and copper of 7.71, 6.61, and 0.74 mg/day may decrease total CVD risk, and also provides reference dose levels for future prospective studies.

Another important factor to consider is the antioxidants ability to prevent CVD relies on the internal antioxidant network mechanism of multiple micronutrients. For example, the fat-soluble vitamins A and E have been evaluated for their synergistic effects on GSH homeostasis and antioxidant properties (55). Vitamin C is known to act with vitamin E to regenerate α-tocopherol in membranes and lipoproteins, playing an important role in protein thiol group protection against oxidation (56). The pro-oxidant vs. antioxidant activity of beta-carotene and lycopene has also been found to depend on their interaction with other co-antioxidant molecules such as vitamin C or E in biological membranes (57). In a mineral antioxidant study, vitamins also showing a protective association between zinc metabolic level influence cardiometabolic risk factor (58). Thus, the single-nutrient approach to nutritional epidemiology is far from sufficient to explain the biological effects of antioxidant micronutrients. The field of nutritional health is shifting toward studying the effects of exposure to combinations of nutrients, and the contribution of its individual components on health outcomes. In this study, WQS regression index showed that the combination of 11 antioxidant micronutrients were negatively associated with CVD. The current study focuses on overall and accurate dietary antioxidant micronutrients data collection, preliminary exploring correlations between individual or combined micronutrients and CVDs. Also found that selenium has the greatest influence on the association between all 11 micronutrients in combination and total CVD.

Selenium is considered a cornerstone of the body's antioxidant defense mechanism; this is because it is incorporated in various enzymes with antioxidant and anti-inflammatory functions. Thirteen prospective cohort (59) and observational studies (60) found a moderate inverse relationship between plasma/serum selenium and CHD (61). A U-shaped relationship between serum selenium levels and cardiovascular mortality may account for conflicting observational reports from NHANES study (62). RCS regression in our study showed linear, negative associations of selenium with both total CVD and specific CVDs (stroke, CHD, CHF, heart attack and angina). Our study investigated the 95th percentiles of dietary selenium intake, which was 207.8 μg/day; large-scale, randomized controlled trials have investigated supplementation with a selenium antioxidant cocktail with a daily dose between 75 and 200 μg or selenium supplementation at 100 μg/day over 12 consecutive months did not show any major benefits of selenium cardiovascular endpoints or left ventricular systolic dysfunction, likely due to underdosage (20).

Our study has some strengths. We selected WQS over alternative approaches in our study because it accounts for exposure-outcome correlations, as well as correlations between exposures, highlighting the contribution of individual components of the combination (63). WQS regression also conserves statistical power and prevents unstable regression coefficients, which might otherwise occur if the highly-correlated antioxidant micronutrients were included simultaneously in traditional regression models. A diet-based, rather than supplement-based, approach to nutritional interventions in CVD had proven to be an effective strategy resulting in strong and tangible results. Micronutrients in the diet have synergistic effects, and incorporating this synergy in the development of dietary recommendations is therefore likely to provide the maximum obtainable benefit obtainable from nutrition.

Our study also has limitations to consider. First, WQS regression requires a directional homogeneity assumption, which assumes that either all exposures have adjusted associations with the outcome that are all in a positive or a negative direction (or can be coded a priori to meet this assumption), or associations are null. However, the antioxidant micronutrients in the study were all shown to be individually negatively associated with CVD, satisfying this assumption. Second, WQS regression also assumes the individual micronutrients have linear and additive effects. Little is known about the benefits of these assumptions and whether they have adverse impacts on studies of epidemiologic data, in which such assumptions can never be met precisely (27). However, this method provides a parsimonious, parametric inference of the effect of combinations of factors, and was therefore a highly appropriate method to use in this study. Third, although the NHANES use of 24-h dietary recall was the preferred choice of reference method. Reliance on memory with twice recall is a well-documented limitation might induce bias for micronutrients calculation. Comprehensive and additional dietary data in NHANES have much more detailed information would be conducive to minimize possibility of self-reporting bias (28). Fourth, although the potential role of the 11 antioxidant micronutrients in the development and progression of CVDs has been investigated, the accuracy of self-reported outcomes in NHANES is not well-characterized. The chemical forms of antioxidant micronutrients (as organic and inorganic compounds) and sustained and dynamic intakes should also be considered. Building on our study, which has demonstrated important synergistic effects of antioxidant micronutrients, randomized trials with standardized antioxidants and protocols would help to improve our understanding of the effects of different food combinations on cardiovascular outcomes.

CONCLUSION

Our findings suggested that higher levels of antioxidative micronutrients in combination are associated with a decreased total CVD risk, and that selenium has the greatest contribution to this effect. Significant negative linear and non-linear correlations exist between the 11 antioxidative micronutrients and total CVD or specific CVDs. Future research is needed to better understand the interactions and complexities of multiple micronutrients and their combined effects on cardiovascular health, and to assess and identify optimal intake levels to reduce CVD in at-risk populations.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TY and XZ designed research, drafted the manuscript, and performed statistical analysis. DX and HL extracted the data and conducted analyses. XLu, YT, and MS took charge of software operation. WY and YZ reviewed the manuscript. XLi and HZ conceptualized the study. All authors reviewed, edited, and finalized the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.799095/full#supplementary-material

Supplementary Figure S1 | Weights from the weighted quantile sum (WQS) regression index for dietary intake of 11 antioxidant micronutrients and the prevalence of total cardiovascular disease (CVD). A protective (negatively-associated) model is shown, adjusted for age, sex, race, education level, smoker status, poverty, body mass index (BMI), and diabetes mellitus status.

Supplementary Figures S2–S6 | Restricted cubic spline (RCS) analysis with a multivariate -adjusted association associations between dietary eleven antioxidant micronutrients and the prevalence of specific CVDs (CHD, CHF, heart attack, angina and stroke). Eleven antioxidant micronutrients index are modeled as restricted cubic splines with knots at the 10th, 50th, and 90th percentiles shown the non-linear association. The solid line is the adjusted HR; 10th percentile is used as the reference (HR = 1). The shaded area is the 95% CI of the HR. The β–cryptoxanthin shown non-linearity association with CHF model (*P* for non-linearity <0.05), and other 10 micronutrients shown linearity correlation with CHF (*P* for non-linearity > 0.05). The iron, zinc and copper shown non-linearity association with heart attack (*P* for non-linearity <0.05), others shown linearity correlation relationship with heart attack (*P* for non-linearity >0.05). All 11 specific antioxidant micronutrients shown linearity association with CHD, angina, and stroke (*P* for non-linearity > 0.05).

Supplementary Table 1 | Concentrations and distribution of the eleven antioxidant micronutrients.

Supplementary Table 2 | Multiple logistic regression model to assess the association between selenium and specific CVD risk.

Supplementary Table 3 | Logistic regression model to assess the association between copper and specific CVD risk.

Supplementary Table 4 | Logistic regression model to assess the association between β-carotene and specific CVD risk.

Supplementary Table 5 | Logistic regression model to assess the association between vitamin E and specific CVD risk.

Supplementary Table 6 | Logistic regression model to assess the association between iron and specific CVD risk.

REFERENCES

1. World Health Organization Report. *Global Atlas on Cardiovascular Disease Prevention and Control*. In: Mendis S, Puska P, Norrving B, editors. Geneva: World Health Organization (2011).
2. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol*. (2017) 70:1–25. doi: 10.1016/j.jacc.2017.04.052
3. Katie LP, Sebastien RS, Alexandre SS. Cardiovascular risk factor mediation of the effects of education and Genetic Risk Score on cardiovascular disease: a prospective observational cohort study of the Framingham Heart Study. *BMJ Open*. (2021) 11:e045210. doi: 10.1136/bmjopen-2020-045210
4. Mensah GA, Roth GA, Fuster V. The global burden of cardiovascular diseases and risk factors: 2020 and beyond. *J Am Coll Cardiol*. (2019) 74:2529–32. doi: 10.1016/j.jacc.2019.10.009
5. Jack S, Katherine A, Sarah C, Peter W. Primary prevention of cardiovascular disease: a review of contemporary guidance and literature. *JRSM Cardiovasc Dis*. (2020) 9:2048004020949326. doi: 10.1177/2048004020949326
6. Batty GD, Kivimäki M, Bell S. Comparison of risk factors for coronary heart disease morbidity versus mortality. *Eur J Prev Cardiol*. (2020) 27:2232–4. doi: 10.1177/2047487319882512
7. Jessica NP, Anita S, Nasab G, Tyler TP, Jennifer QK. Mitochondrial dysfunction and oxidative stress in heart disease. *Exp Mol Med*. (2019) 51:162. doi: 10.1038/s12276-019-0355-7
8. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. *Curr Atheroscler Rep*. (2017) 19:42. doi: 10.1007/s11883-017-0678-6
9. Shah AK, Bhullar SK, Elimban V, Dhalla NS. Oxidative stress as a mechanism for functional alterations in cardiac hypertrophy and heart failure. *Antioxidants (Basel)*. (2021) 10:931. doi: 10.3390/antiox10060931
10. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. *Am J Med*. (1994) 97:5S−13S. doi: 10.1016/0002-9343(94)90292-5
11. Minich DM, Benjamin I. Brown a review of dietary (phyto)nutrients for glutathione support. *Nutrients*. (2019) 11:2073. doi: 10.3390/nu11092073
12. Sánchez-Hernández D, Anderson GH, Poon AN, Pannia E, Cho CE, Huot PSP, et al. Maternal fat-soluble vitamins, brain development, and regulation of feeding behavior: an overview of research. *Nutr Res*. (2016) 36:1045–54. doi: 10.1016/j.nutres.2016.09.009
13. Fortmann SP, Burda BU, Senger CA, Lin JS, Whitlock EP. Vitamin and mineral supplements in the primary prevention of cardiovascular disease and cancer: An updated systematic evidence review for the US Preventive Services Task Force. *Ann Intern Med*. (2013) 159:824–34. doi: 10.7326/0003-4819-159-12-201312170-00729
14. Kulczyński B, Gramza-Michałowska A, Kobus-Cisowska J, Kmiecik D. The role of carotenoids in the prevention and treatment of cardiovascular disease—Current state of knowledge. *J Funct Foods*. (2017) 38:45–65. doi: 10.1016/j.jff.2017.09.001
15. Toti E, Chen CO, Palmery M, Villaño Valencia D, Peluso I. Non-Provitamin A and provitamin A carotenoids as immunomodulators: recommended dietary allowance, therapeutic index, or personalized nutrition? *Oxid Med Cell Longev*. (2018) 2018:4637861. doi: 10.1155/2018/4637861
16. Schwingshackl L, Boeing H, Stelmach-Mardas M, Gottschald M, Dietrich S, Hoffmann G, et al. Dietary supplements and risk of cause-specific death, cardiovascular disease, and cancer: a systematic review and meta-analysis of primary prevention trials. *Adv Nutr*. (2017) 8:27–39. doi: 10.3945/an.116.013516
17. Knekt P, Ritz J, Pereira MA, O'Reilly EJ, Augustsson K, Fraser GE, et al. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am J Clin Nutr*. (2004) 80:1508–20. doi: 10.1093/ajcn/80.6.1508
18. Loffredo L, Perri L, Di Castelnuovo A, Iacoviello L, De Gaetano G, Violi F. Supplementation with vitamin E alone is associated with reduced myocardial infarction: a meta-analysis. *Nutr Metab Cardiovasc Dis*. (2015) 25:354–63. doi: 10.1016/j.numecd.2015.01.008
19. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. Selenium and coronary heart disease: a meta-analysis. *Am J Clin Nutr*. (2006) 84:762–73. doi: 10.1093/ajcn/84.4.762
20. Raygan F, Behnejad M, Ostadmohammadi V, Bahmani F, Mansournia MA, Karamali F, et al. Selenium supplementation lowers insulin resistance and markers of cardio-metabolic risk in patients with congestive heart failure: a randomised, double-blind, placebo-controlled trial. *Br J Nutr*. (2018) 120:33–40. doi: 10.1017/S0007114518001253
21. Pilz S, Dobnig H, Winklhofer-Roob BM, Renner W, Seelhorst U, Wellnitz B, et al. Low serum zinc concentrations predict mortality in patients referred to coronary angiography. *J Nutr*. (2009) 101:1534–40. doi: 10.1017/S0007114508084079
22. Vittoria C, Cristina N, Simona B, Valerio S, Davide F, Sebastiano S, et al. The role of antioxidants supplementation in clinical practice: focus on cardiovascular risk factors. *Antioxidants (Basel)*. (2021) 10:146. doi: 10.3390/antiox10020146
23. Bates CJ, Hamer M, Mishra GD. Redox-modulatory vitamins and minerals that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over. *Br J Nutr*. (2011) 105:123–32. doi: 10.1017/S0007114510003053
24. Ye Y, Li J, Yuan Z. Effect of antioxidant vitamin supplementation on cardiovascular outcomes: a meta-analysis of randomized controlled trials. *PLoS ONE*. (2013) 8:e56803. doi: 10.1371/journal.pone.0056803
25. Sherry AT, Robert MR, Charles BS, Bryan MG, Neal EC, Marjorie JH, et al. Biomarkers of nutrition for development (BOND)—vitamin A review. *J Nutr*. (2016) 146:1816S—48S. doi: 10.3945/jn.115.229708
26. Hamra GB, Buckley JP. Environmental exposure mixtures: questions and methods to address them. *Curr Epidemiol Rep*. (2018) 5:160–5. doi: 10.1007/s40471-018-0145-0
27. Alexander PK, Jessie PB, Katie MO, Kelly KF, Shanshan Z, Alexandra JW, et al. Quantile-based g-computation approach to addressing the effects of exposure mixtures. *Environ Health Perspect*. (2020) 128:047004. doi: 10.1289/EHP5838
28. Paulose-R R, Burt V, Broitman L, Ahluwalia N. Overview of asian american data collection, release, and analysis: national health and nutrition examination survey 2011–2018. *Am J Public Health*. (2017) 107:916–21. doi: 10.2105/AJPH.2017.303815
29. Charles FD, Michael HW, Frederick WM. Population-based estimates of humoral autoimmunity from the U.S. National Health and Nutrition Examination Surveys, 1960–2014. *PLoS ONE*. (2020) 15:e0226516. doi: 10.1371/journal.pone.0226516
30. Zipf G, Chiappa M, Porter K, Ostchega Y, Lewis B, Dostal J. The National Health and Nutrition Examination Survey: plan and operations, 1999–2010. *Vital Health Stat 1*. (2013) 56:1–37.
31. Namanjeet A. Nutrition monitoring of children aged birth to 24 Mo (B-24): data collection and findings from the NHANES. *Adv Nutr*. (2020) 11:113–27. doi: 10.1093/advances/nmz077
32. Elisabeth LPS, Yuta I, Rupal TK, Donglan Z, Arshed AQ, Sandra BD. Association between the prognostic nutritional index and dietary intake in community-dwelling older adults with heart failure: findings from NHANES III. *Nutrients*. (2019) 11:2608. doi: 10.3390/nu11112608
33. Liyun C, Qianwen L, Xuexian F, Xinhui W, Junxia M, Fudi W. Dietary intake of homocysteine metabolism-related B-vitamins and the risk of stroke: a dose-response meta-analysis of prospective studies. *Adv Nutr*. (2020) 11:1510–28. doi: 10.1093/advances/nmaa061
34. Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. *J Agric Biol Environ Stat*. (2015) 20:100–20. doi: 10.1007/s13253-014-0180-3
35. Han C, Hong YC, Bisphenol A. Hypertension, and cardiovascular diseases: epidemiological, laboratory, and clinical trial evidence. *Curr Hypertens Rep*. (2016) 18:11. doi: 10.1007/s11906-015-0617-2
36. GBD 2013. Mortality and Causes of Death Collaborators Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. (2015) 385:117–71. doi: 10.1016/S0140-6736(14)61682-2
37. Bhaskaran K, Dos-Santos-Silva I, Leon DA, Douglas IJ, Smeeth L. Association of BMI with overall and cause-specific mortality: a population-based cohort study of 3·6 million adults in the UK. *Lancet Diabetes Endocrinol*. (2018) 6:944–53. doi: 10.1016/S2213-8587(18)30288-2
38. Rossella D'O, Rossella S, Anna L, Annalisa N, Sebastio P, Angelo C, et al. The role of oxidative stress in cardiac disease: from physiological

response to injury factor. *Oxid Med Cell Longev*. (2020). 2020:5732956. doi: 10.1155/2020/5732956
39. William M, Rupinder D, Xuran J, Lauren M, Daren K H. Antioxidant micronutrients in the critically ill: a systematic review and meta-analysis. *Crit Care.* (2012) 16:R66. doi: 10.1186/cc11316
40. Meganathan P, Fu JY. Biological properties of tocotrienols: evidence in human studies. *Int J Mol Sci.* (2016) 17:1682. doi: 10.3390/ijms17111682
41. De Waart FG, Moser U, Kok FJ. Vitamin E supplementation in elderly lowers the oxidation rate of linoleic acid in LDL. *Atherosclerosis.* (1997) 133:255–63. doi: 10.1016/S0021-9150(97)00137-8
42. Berger MM. Can oxidative damage be treated nutritionally? *Clin Nutr.* (2005) 16:172–83. doi: 10.1016/j.clnu.2004.10.003
43. Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. *Ann Rev Nutr.* (2005) 25:151–74. doi: 10.1146/annurev.nutr.24.012003.132446
44. Reunanen A, Knekt P, Marniemi J, Mäki J, Maatela J, Aromaa A. Serum calcium, magnesium, copper and zinc and risk of cardiovascular death. *Eur J Clin Nutr.* (1996) 50:431–7.
45. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the physicians' health study II randomized. *Trial JAMA.* (2008) 300:2123–33. doi: 10.1001/jama.2008.600
46. Koh KK, Blum A, Hathaway L, Mincemoyer R, Csako G, Waclawiw MA, el at. Vascular effects of estrogen and vitamin E therapies in postmenopausal women. *Circulation*. (1999) 100:1851–7. doi: 10.1161/01.CIR.100.18.1851
47. Lee DH, Folsom AR, Jacobs DR, Jr. Iron, zinc, and alcohol consumption and mortality from cardiovascular diseases: the Iowa Women's Health Study. *Am J Clin Nutr.* (2005) 81:787–91. doi: 10.1093/ajcn/81.4.787
48. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta analysis of randomised trials. *Lancet.* (2003) 361:2017–2. doi: 10.1016/S0140-6736(03)13637-9
49. Ergul BK. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J.* (2016) 15:71. doi: 10.1186/s12937-016-0186-5
50. MacFarquhar JK, Broussard DL, Melstrom P, Hutchinson R, Wolkin A, Martin C, et al. Acute selenium toxicity associated with a dietary supplement. *Arch Intern Med.* (2010) 170:256–61. doi: 10.1001/archinternmed.2009.495
51. Alpha T. Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med.* (1994) 330:1029–35. doi: 10.1056/NEJM199404143301501
52. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, et al. Fruit and vegetable intake in relation to risk of ischemic stroke. *JAMA.* (1999) 282:1233–9. doi: 10.1001/jama.282.13.1233
53. Hoffmann FW, Hashimoto AS, Lee BC, Rose AH, Shohet RV, Hoffmann PR. Specific antioxidant selenoproteins are induced in the heart during hypertrophy. *Arch Biochem Biophys.* (2011) 512:38–44. doi: 10.1016/j.abb.2011.05.007
54. Sabate J. Nut consumption, vegetarian diets, ischemic heart disease risk, and all-cause mortality: evidence from epidemiologic studies. *Am J Clin Nutr*. (1999) 70(Suppl. 3):S500–3. doi: 10.1093/ajcn/70.3.500s
55. Heyland DK, Dhaliwal R, Suchner U, Berger MM. Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient. *Intensive Care Med.* (2004) 31:327–37. doi: 10.1007/s00134-004-2522-z
56. Wang X, Quinn PJ. The location and function of vitamin E in membranes. *Mol Membr Biol.* (2000) 17:143–56. doi: 10.1080/09687680010000311
57. Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys.* (2001) 385:20–7. doi: 10.1006/abbi.2000.2149
58. Czernichow S, Vergnaud AC, Galan P, Arnaud J, Favier A, Faure H, et al. Effects of long-term antioxidant supplementation and association of serum antioxidant concentrations with risk of metabolic syndrome in adults. *Am J Clin Nutr.* (2009) 90:329–35. doi: 10.3945/ajcn.2009.27635
59. Navas-Acien A, Bleys J, Guallar E. Selenium intake and cardiovascular risk: what is new? *Curr Opin Lipidol.* (2008) 19:43–9. doi: 10.1097/MOL.0b013e3282f2b261
60. Xun P, Liu K, Morris JS, Daviglus ML, He K. Longitudinal association between toenail selenium levels and measures of subclinical atherosclerosis: the CARDIA trace element study. *Atherosclerosis.* (2010) 210:662–7. doi: 10.1016/j.atherosclerosis.2010.01.021
61. Joseph J. Selenium and cardiometabolic health: inconclusive yet intriguing evidence. *Am J Med Sci.* (2013) 346:216–20. doi: 10.1097/MAJ.0b013e3182638716
62. Bleys J, Navas-Acien A, Guallar E. Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. *Arch Intern Med.* (2008) 168:404–10. doi: 10.1001/archinternmed.2007.74
63. Czarnota J, Gennings C, Wheeler DC. Assessment of weighted quantile sum regression for modeling chemical mixtures and cancer risk. *Cancer Inform.* (2015) 14:159–71. doi: 10.4137/CIN.S17295

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REVIEW
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Effects of Resistant Starch Interventions on Metabolic Biomarkers in Pre-Diabetes and Diabetes Adults

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Simple lifestyle changes can prevent or delay the onset of type 2 diabetes mellitus (T2DM). In addition to maintaining a physically active way of life, the diet has become one of the bases in managing TD2M. Due to many studies linking the ability of resistant starch (RS) to a substantial role in enhancing the nutritional quality of food and disease prevention, the challenge of incorporating RS into the diet and increasing its intake remains. Therefore, we conducted this review to assess the potential benefits of RS on metabolic biomarkers in pre-diabetes and diabetes adults based on available intervention studies over the last decade. Based on the conducted review, we observed that RS intake correlates directly to minimize possible effects through different mechanisms for better control of pre-diabetic and diabetic conditions. In most studies, significant changes were evident in the postprandial glucose and insulin incremental area under the curve (iAUC). Comparative evaluation of RS consumption and control groups also showed differences with inflammatory markers such as TNF-α, IL-1β, MCP-1, and E-selectin. Only RS2 and RS3 were extensively investigated and widely reported among the five reported RS types. However, a proper comparison and conclusion are deemed inappropriate considering the variations observed with the study duration, sample size, subjects and their metabolic conditions, intervention doses, and the intervention base products. In conclusion, this result provides interesting insights into the potential use of RS as part of a sustainable diet in diabetes management and should be further explored in terms of the mechanism involved.

Keywords: resistant starch, type 2 diabetes, biomarkers, glucose, insulin

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defective insulin secretion, action or both (1). The diagnostic criteria for diabetes include fasting plasma glucose (FPG) ≥7.0 mmol/L (2), glycated hemoglobin (HbA1c) ≥6.5% (2–4), 2-h plasma glucose (2hPG) in a 75 g oral glucose tolerance test (OGTT) ≥11.1 mmol/L (2) or random PG ≥11.1 mmol/L. Meanwhile, pre-diabetes refers to impaired fasting glucose (IFG) of 6.1–6.9 mmol/L (5), impaired glucose tolerance (IGT)/2hPG in a 75 g OGTT of 7.8–11.0 mmol/L (5) or HbA1c of 6.0% to 6.4% (6), each of which places individuals at high risk of developing diabetes and its complications (7).

A recent study by Khan and his team (8) presented the epidemiology of type 2 diabetes mellitus (T2DM) in terms of the global burden of disease and forecasted trends. The study showed that T2DM continues to be the leading cause of human suffering and deaths as it continues to increase in prevalence and incidence. This phenomenon continues, and there are no sights for reduction despite efforts in clinical care, research and public health interventions (8). Globally, it was recognized that diets low in whole grains, nuts, seeds and fruits were the leading dietary risks, especially in developing countries where globalization and emerging supermarkets increase access to processed, salt-laden, high-fat and sugar-added food products. Consequently, the attractiveness in line with low prices and higher accessibility led to decreased intake of whole grains, fruits and vegetables. In addition, poor dietary control and a sedentary lifestyle contribute to higher BMI that further elevates the associated risks (9).

The nutritional recommendation based on FAO/WHO states that the acceptable macronutrient distribution range should be within 55–75% of net energy for carbohydrates, 10% for sugar components, while dietary fiber intake requirements differ with 38 g and 25 g for men and women, respectively (10). Several published findings have highlighted the association of high fiber and grains intake toward lower risk of both obesity and diabetes. However, the usual dietary pattern does not meet the recommended quantity, and hence it was suggested to include an additional grain-fibrous food for a more enhanced approach in managing metabolic conditions, such as diabetes (11–13).

Starch is the predominant form of carbohydrate and is categorized as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) determined based on their digestion rate. SDS seems to be slowly digested within the small intestines, whereas RDS tends to elevate glucose levels rapidly in the blood upon consumption. On the other hand, RS is undigested in the small intestines and usually fermented in the colon (14). Several studies have shown that RS is a linear molecule of α-1,4-D-glucan, derived from the retrograded amylose fraction and has a relatively low molecular weight (1.2 $\times 10^5$ Da) (15, 16). The chemical structure of α-1,4-D-glucan is shown in **Figure 1** (17).

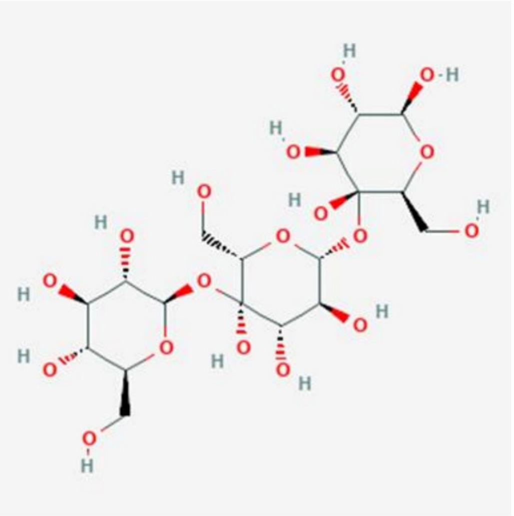

FIGURE 1 | Chemical structure of α-1,4-D-glucan [Obtained from: (17)].

RS is used widely as a functional ingredient, especially in foods containing high dietary fiber levels. These types of food are assumed to help prevent several pathologies such as colon cancer, diabetes, or obesity (18). It has been proposed that foods containing naturally occurring RS or foods modified to contain more RS may alter the postprandial glycemic response, prevent hypoglycemia, reduce hyperglycemia and explain differences in some foods' glycemic index (GI). Dietary supplementation of RS is one of the nutritional interventions practiced for metabolic disease (19). Bread, cooked cereals and pasta, vegetables, just-ripe bananas are several familiar food products that serve as natural as natural RS sources. Consumption of RS foods tends to resist digestion in the small intestines and reaches the large intestines for microbial fermentation (14, 20, 21).

There are five types of RS based on their indigestible properties (RS1, RS2, RS3, RS4, and RS5) (16, 17). RS1 refers to physically entrapped starch within whole plant cells and food matrices (e.g., partly milled grains and seeds) where there is a physical barrier to amylolysis. The presence of intact cell walls contributes to the RS content of legumes. Extensive milling (and chewing) allows these starches to be more accessible and less resistant. RS2 comprises the poorly gelatinized and hydrolyzed granules by α-amylases from certain plants (e.g., raw potato and green banana, HAM). Retrograded starches constitute RS3, which includes cooked and cooled rice or potatoes. Meanwhile, RS4 is a group of chemically modified starches that can improve the functional characteristics of the starch. Amylose-lipid complex has been proposed as RS5 because high-amylose starch tends to be more resistant to enzyme hydrolysis than low-amylose

Abbreviations: Aβ, Amyloid-beta; AD, Adzuki bean; ACh, Acetylcholine; AChE, Acetylcholinesterase; AD, Alzheimer's disease; AFM, Atomic Force Microscope; BMI, Body mass index; BR, Waxy black rice; CHO, Carbohydrate; CRP, C-reactive protein; CSIRO, Commonwealth Scientific and Industrial Research Organization; ELD, Elderly; FFA, Free Fatty acid; FPG, Fasting Plasma Glucose; FSIVGTT, Frequently sampled intravenous glucose tolerance test; GI, Glycemic index; GLP1, Glucagon-like peptide-1; IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; iAUC, Incremental area under the curve; IR, Insulin resistance; IL, Interleukin; IN, Inulin; HbA1c, Glycated hemoglobin; HAM, High-amylose maize; HAW, High-amylose wheat; HDL, High-density lipoprotein; HFWR, High fiber white rice; hs, High-sensitive; LAW, Low-amylose wheat; LB, Lily bulb; LDL, Low-density lipoprotein; LN, Lean; LS, Lotus seed; MCP-1, Monocyte chemotactic protein-1; MI, Millet; MID, Mid-age; MISI, Muscle insulin sensitivity index; MTT, Meal tolerance test; NEFA, Non-esterified fatty acids; OGTT, Oral glucose tolerance test; OWO, Overweight or obese; PAI-1, Plasminogen activator inhibitor-1; PYY, Peptide YY; RB, Rye-based bread; RDS, Rapidly digestible starch; RFB, Rye flour bread; RKB, Rye kernels bread; RS, Resistant starch; SCFA, Serum short-chain FA; SDS, slowly digestible starch; TC, Total cholesterol; TEE, Total energy expenditure; TG, Triglycerides; T2DM, Type 2 diabetes; sVCAM-1, Soluble vascular cell adhesion molecule −1; WG, Whole grain rye; WR, White rice; WWB, Wheat flour bread; 2hPG, 2-h plasma glucose.

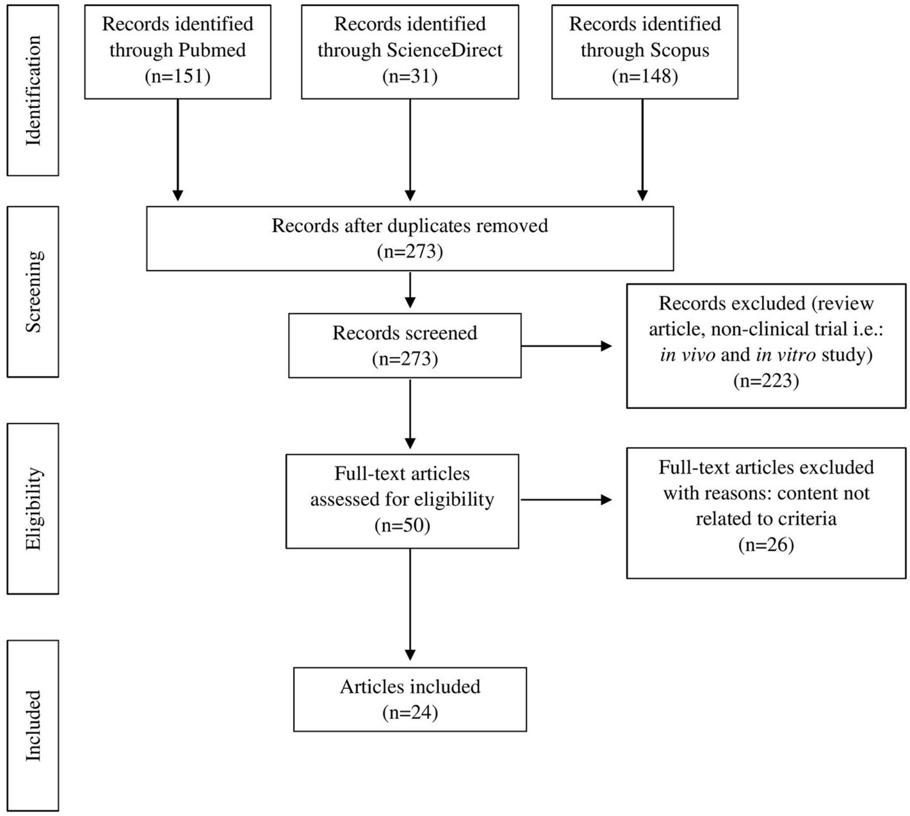

FIGURE 2 | PRISMA flow diagram of literature search and selection process.

starch. The amylose-lipid complex in starch granules increases their enzyme resistance by restricting the granule swelling during cooking. Although these modified starches are found widely in processed foods, neither their contribution to RS intakes nor their physiological effects have been extensively studied (22).

RS has the advantage of having a less negative influence on the sensory properties of final products than traditional fibers, such as whole grains, bran or fruit fibers, which is very positive for consumer acceptability (23). Consumption of RS has resulted in changes in insulin sensitivity, IGT and satiety in healthy humans (24) and therefore has been hypothesized to have implications for glycemic control in individuals at risk of or with T2DM. Among the five types, RS2 and RS3 were the most common form utilized for interventions, especially in evaluating their effects on blood lipids, GI and colon cancer (24, 25). RS-based diets are one of the nutritional interventions practiced for metabolic disease control, and these include common food sources such as bread, cooked cereals and pasta, vegetables and just-ripe bananas. Despite the numerous interventions on evaluating RS effects on diabetic control, the findings seem to be limited to short-term studies and lack longer-term studies to prove the benefits of RS. Thus, in this review, we intend to explore the reported studies on RS diet interventions to further understand its importance as well as to investigate the possible effects exerted by various RS-based intervention studies among pre-diabetes and diabetes adults.

MATERIALS AND METHODS

Search Strategy

Original articles were searched in three databases (PubMed, Scopus and ScienceDirect) from the year 2011 to 2021 using the Medical subject heading (MeSH) terms "diabetes," crossed with the term "resistant starch." Publications with available abstracts were reviewed and limited to studies published in English. Papers on human and clinical trials related to diabetes were included. However, review articles, proceedings, letters to the editor, and *in vitro* and *in vivo* studies were excluded. Duplicate articles were eliminated. The study identification process and reasons for exclusion are illustrated in **Figure 2**.

Eligibility Criteria

We included published intervention studies (defined as a randomized controlled trial, crossover study and quasi-experimental study) comparing markers of glucose metabolism for RS consumption. We included only human studies with adult participants aged at least 18 years old from both genders. Studies were included if they analyzed at least one of the biomarkers as mentioned earlier.

Study Selection

A pair of authors independently assessed the titles and abstracts during the initial screening. The difference in the initial assessment was resolved by a discussion leading to a consensus, with a third party serving as arbitrator if necessary. Each study was recorded as include, exclude or unclear. Full articles were retrieved for further assessment if recorded as include or unclear. Any disagreement was re-evaluated and re-assessed among the reviewers.

RESULTS

The search resulted in 50 articles produced with a refined search based on the availability of full text, peer-reviewed articles and library collection access. Upon further assessment, only 24 full-text articles were relevant and included for final review (**Table 1**). All the related articles were printed out for further assessment of evidence-based to explore the effectiveness of RS as a potential antidiabetic agent.

DISCUSSION

Table 1 was constructed based on available intervention studies on RS over the last decade. Although there are five types of RS, only three types have been clinically studied based on our search strategies from the three selected databases. HAM-RS2, an ingredient available to both food producers and consumers, was the usual form of RS2 used. HAM-RS2 is a bland, white substance with a small particle size comprised of 60% RS and 40% SDS (50). A randomized crossover study done by Bodinham and her colleagues on 12 overweight individuals reported significantly higher plasma insulin and C-peptide concentrations in individuals who consumed 40 g/d RS2 for 4 wks compared with the placebo (27). The process of insulin synthesis involves cleaving stages of C-peptide from proinsulin and is stored within secretory granules before its release in equimolar amounts with insulin into the bloodstream. C-peptide plays a vital role during this process by linking the A and B chains leading to precise folding and formation of interchain disulphide bonds (51). The presence of a higher level of both insulin and C-peptide assists the cells for better glucose absorption, blood sugar reduction and channeling glucose to the cells for energy synthesis. It is important to note that the circulating venous (or arterial) fasting insulin concentrations are about 18–90 pmol/L in healthy LN individuals (52). However, no changes were reported to either bodyweight or habitual food intake of the subjects in the study by Bodinham and team (26).

In another crossover study done by the same group of researchers on individuals with well-controlled T2DM, RS2 consumption resulted in significantly lower postprandial glucose concentrations ($p < 0.05$) without any effect on hepatic, peripheral insulin sensitivity or HbA1c levels (27). Several biochemical abnormalities were observed in insulin and glucagon secretion, uptake and suppression of hepatic glucose production, and uptake of peripheral glucose among diabetic individuals that led to higher and prolonged postprandial glucose (PPG) excursions when compared with non-diabetic individuals (53). Generally, PPG accounted for ∼80% of HbA1c when HbA1c was <6.2% and only about 40% when HbA1c was above 9.0% (54). Decreases in PPG accounted for nearly twice as much for the reduction in HbA1c as did the decline in FPG. HbA1c values reflect overall glycemic exposure over the past 2-3 mths integrating both FPG and PPG levels.

Despite no improvement effect of HAM-RS2 on tissue insulin sensitivity in well-controlled T2DM, it demonstrated beneficial effects on meal handling, possibly due to higher postprandial glucagon-like peptide-1 (GLP1). GLP-1 delays gastric emptying and gut motility in healthy LN and obese subjects and patients with T2DM (55, 56). GLP-1 also contributes to the change in gastric volume that occurs in anticipation of food ingestion (57). In contrast, a double-blind crossover study done by Dainty and her colleagues in adults at an increased risk of T2DM characterized by a Canadian diabetes risk assessment questionnaire (CANRISK) score ≥21 (58) found that consumption of one bagel containing 25 g/d HAM-RS2 for 8 wks did not show any differences in FPG and PPG between the RS-intervention and control. However, the HAM-RS2 bagel recorded significantly lower fasting insulin iAUC and fasting IR (28). These findings are consistent with the results of another study done by Galarregui and co-workers that found positive associations of both fasting insulin and HOMA-IR with the insulin iAUC for foods with varied macronutrient composition consumed by senior participants (59). Interestingly, an investigation by Peterson and co-investigators (29) also recorded no improvement in glycemic control, cardiovascular disease risk factors and energy metabolism relative to baseline when pre-diabetic adults consumed HAM-RS2 for 12 wks. The sole exception was a decrease in circulating concentrations of TNF-α.

A possible explanation for the inconsistent findings of RS2 is partly because of the underlying dietary variability among individuals who participated in the studies. As mentioned earlier, RS2 supplementation did not improve cardiometabolic health in adults with pre-diabetes, although it does reduce TNF-α concentrations (29). Thus, it may be necessary to search for any relevant articles that correlate various factors in terms of gut microbiota, diet composition, biological and environmental factors that respond better to RS2 supplementation than others. For example, the possible mechanism in the management of T2DM via activation of protein phosphatase-4, TNF-α and plasminogen-activator inhibitors were reported in several studies (60, 61). These articles could lead to a better understanding of the potential beneficial effects of RS supplementation on metabolic health and whether such effects are modulated by diet compositions or existing microbial populations in the gut.

In addition, increasing evidence relates the roles of gut microbiota with T2DM development (62). Diabetic developments are also affected by chemical and diet-related factors (63). Recently, RS2 in the form of green-banana biomass studied in 39 T2DM patients of both genders for 6 mths showed significantly lower TC, non-HDL-cholesterol, glucose, and HbA1c levels ($p < 0.05$), as well as improved the LDL particles

TABLE 1 | The potential health benefit effect of RS interventions on diabetic biomarkers.

References	Objective (s)	Methods	Findings	Conclusion
Bodinham et al. (26)	To further explore the effects of RS on insulin secretion.	• A subject-blind, randomized crossover study. • 12 overweight individuals (37 ± 4.0 yrs) consumed either 40 g RS2 or energy and carbohydrate (CHO)-matched placebo daily, for 4 wks. • Assessment of insulin secretion, plasma insulin and C-peptide concentrations.	• Significantly higher plasma insulin and C-peptide concentrations with RS ($p < 0.05$). • Significantly improved 1st phase insulin secretion with RS ($p < 0.05$). • No changes on body weight or habitual food intake.	• RS intake significantly increased the 1st -phase insulin secretion in individuals at risk of developing T2DM. • Further studies exploring this effect in individuals with T2DM are required.
Bodinham et al. (27)	To determine the effects of increased RS consumption on insulin sensitivity and glucose control and changes in postprandial metabolites and body fat in T2DM.	• A single-blind, randomized dietary intervention crossover study. • 17 individuals (mean age 55 yrs) with well-controlled T2DM consumed either 40 g of HAM-RS2 or placebo for 12 wks. • Three metabolic investigations: a two-step euglycemic–hyperinsulinemic clamp combined with an infusion of [6,6-$^{2}H_2$] glucose, a meal tolerance test (MTT) with arterio-venous sampling across the forearm, and whole-body imaging. • Determination of plasma glucose, insulin, triglycerides (TG), non-esterified fatty acids (NEFA), total cholesterol (TC) and high-density lipoprotein (HDL). • Determination of fasting tumor necrosis factor-α (TNF-α) and interleukin (IL) 6, C-peptide and total glucagon-like peptide-1 (GLP1).	• Significantly lower postprandial glucose concentrations ($p = 0.045$). • No effect of HAM-RS2 on hepatic, peripheral insulin sensitivity, or HbA1c. • No significant difference in C-peptide, HOMA, TC, HDL and IL6 between the HAM-RS2 and placebo. • Significant difference in NEFA, TG and TNF-α. • Fasting GLP1 concentrations were significantly lower following HAM-RS2 consumption ($p = 0.049$). • Significantly greater postprandial GLP1 excursions during the MTT ($p = 0.009$).	• HAM-RS2 did not improve tissue insulin sensitivity in well-controlled T2DM, but demonstrated beneficial effects on meal handling, possibly due to higher postprandial GLP1.
Dainty et al. (28)	To examine the chronic effects of consuming bagels high in HAM-RS2 on fasting and postprandial glycemic markers in adults at increased risk of T2DM.	• A randomized, double-blind crossover design. • 24 men and women (mean age of 55.3 ± 1.59 yrs and body mass index (BMI) of 30.2 ± 0.57 kg/m^2) consumed 1 bagel containing 25 g/d HAM-RS2 or one control wheat bagel for 56 d each, separated by a 4 wks washout. • Fasting and postprandial OGTT glucose and insulin.	• Significantly lower fasting, 2 h and 3 h insulin incremental area under the curve (iAUC) and fasting insulin resistance (IR) than control ($p < 0.05$). • No difference in fasting and postprandial OGTT glucose concentrations.	• Consumption of a high-HAM-RS2 bagel improves glycemic efficiency and fasting insulin sensitivity in adults at increased risk of T2DM.
Peterson et al. (29)	To test whether RS2 can improve cardiometabolic health among pre-diabetic adults.	• A randomized, double-blind, placebo-controlled, parallel-arm trial. • 68 overweight adults (35–75 yrs) with pre-diabetes were randomized to consume 45 g/d of HAM-RS2 or an isocaloric amount of the RDS amylopectin (control) for 12 wks. • HbA1c, insulin sensitivity, insulin secretion, ectopic fat, and markers of inflammation.	• RS2 lowered HbA1c by a clinically insignificant ($p > 0.05$). • RS2 also did not affect insulin sensitivity, TG, TC, FFA, high-sensitive (hs)-CRP, iAUC relative to baseline ($p > 0.05$). • Significant reduction in TNF-α, and heart rate ($p < 0.05$).	• RS supplementation reduced the inflammatory marker TNF-α and heart rate, but it did not significantly improve glycemic control and other cardiovascular disease risk factors among pre-diabetic adults.

(Continued)

TABLE 1 | Continued

References	Objective (s)	Methods	Findings	Conclusion
Kwak et al. (30)	To evaluate whether 4 wks of dietary treatment with rice containing RS reduces blood glucose and oxidative stress as well as improves endothelial function.	• Patients with IFG, IGT or newly diagnosed T2DM ($n = 90$) were randomly assigned to either rice containing 6.51 g RS/d or a control rice group for 4 wks. • Fasting and postprandial levels of glucose and insulin, oxidative stress markers and endothelial function.	• Significant reduction on fasting insulin and IR, postprandial glucose ($p < 0.010$) and insulin levels at 30 min, and glucose and iAUC after the standard meal. • Decreased urinary 8-epi-$PGF_{2\alpha}$ and plasma malondialdehyde (MDA) and increased the RH-PAT index ($p < 0.001$) and total nitric oxide (NO). • Postprandial changes in glucose at 60 and 120 min and areas under the glucose response curve, MDA, RH-PAT, and total NO of the test group differed significantly from control.	• In patients with IFG, IGT or newly diagnosed T2DM, rice containing RS was associated with improved endothelial function reduction of postprandial glucose and oxidative stress compared with control.
Lotfollahi et al. (31)	To investigate the effects of 6 mths consumption of green-banana biomass on the LDL particle functionality in subjects with T2DM.	• Subjects ($n = 39$, mean age 65 yrs) of both sexes with diabetes (HbA1c $\geq$ 6.5%) were randomized to receive nutritional support plus green-banana biomass (40 g) ($n = 21$) or diet alone ($n = 18$) for 6 months. • Non-linear optical responses of LDL solutions from these participants were studied by Z-scan technique. • Measurement absorbance structural changes in LDL samples and determination of LDL sub-fractions.	• Significant reduction on total- and non-HDL-cholesterol, glucose, HbA1c and improved the protection of the LDL particle against oxidation, by increasing carotenoids content in the particles ($p < 0.05$).	• Higher protection against modifications may decrease the risk of developing cardiovascular disease. • Benefits of the green-banana biomass encourage the RS usage with potential clinical applications among pre-diabetic and diabetic individuals.
Gargari et al. (32)	To determine effects of RS2 on metabolic parameters and inflammation in women with T2DM.	• A randomized controlled clinical trial. • 60 females (30–65 yrs) with T2DMreceived 10 g/d RS2 or placebo for 8 wks. • FPG, HbA1c, lipid profile, hs-CRP, IL-6 and TNF-α.	• RS2 significantly decreased HbA1c (−0.3%, −3.6%), TNF-α (−3.4 pg/mL, −18.9%) compared with placebo ($p < 0.05$). • Changes in FBS, hs-CRP and IL-6 were not significant.	• RS2 can improve glycemic status, inflammatory markers and lipid profile in women with T2DM. • More studies are needed to confirm efficacy of RS2 as an adjunct therapy in diabetes.
Alfa et al. (33)	To determine the tolerability as well as the glucose and insulin modulating ability of *MSPrebiotic*® digestion RS in healthy mid-age (MID) and elderly (ELD) adults.	• A prospective, blinded, placebo-controlled study. • ELD (>70 yrs) and MID (30–50 yrs) consumed either 30 g/d *MSPrebiotic*® or placebo for 12 wks. • Blood glucose, lipid profile, C-reactive protein (CRP), lipid particles, TNF-α, IL-10, insulin and IR.	• A significant difference in blood glucose ($p = 0.0301$) and insulin levels ($p = 0.009$), as well as IR (HOMA-IR; $p = 0.009$) in ELD adults who consumed *MSPrebiotic*. • *MSPrebiotic*® consumption for 12 wks was not sufficient to reduce the elevated CRP and TNF-α levels in the ELD group. • No significant changes in MID adults.	• Dietary supplementation with prebiotics such as *MSPrebiotic*® may be part of an effective strategy to reduce IR, in the ELD.
Giles et al. (34)	To determine the *in vivo* net energy content of RS and examine its effect on macronutrient oxidation.	• A randomized, double-blind cross-over study. • 18 healthy adults aged 25–45 yrs. • Measurement of total energy expenditure (TEE), substrate oxidation, and postprandial metabolites in response to three diets: (a) digestible starch (DS), (b) RS (33% dietary fiber), (c) RS with high fiber (RSF, 56% fiber).	• The in vivo net energy content of RS and RSF are 2.74 ± 0.41 and 3.16 ± 0.27 kcal/g, respectively. • No difference in TEE or protein oxidation between DS, RS, and RSF. • RS and RSF consumption caused a 32% increase in fat oxidation ($p = 0.04$) with a concomitant 18% decrease in CHO oxidation ($p = 0.03$) vs. DS. • Insulin responses were unaltered after breakfast but lower in RS and RSF after lunch, at equivalent glucose concentrations.	• RS and RSF consumption increase fat and decrease CHO oxidation with postprandial insulin responses lowered after lunch.

(Continued)

TABLE 1 | Continued

References	Objective (s)	Methods	Findings	Conclusion
Belobrajdic et al. (35)	To determine if bread made from HAW and enriched in RS dampens postprandial glycemia compared with bread made from conventional low-amylose wheat (LAW).	• A single-center, randomized, double-blinded, crossover- controlled study. • 20 healthy non-diabetic men and women (mean age 30 ± 3 yrs; BMI 23 ± 0.7 kg/m^2) consumed a glucose beverage or 4 different breads (LAW-R (refined), LAW-W (wholemeal), HAW-R, or HAW-W) for 7 wks. • Plasma glucose, insulin, ghrelin, incretin hormone concentrations and iAUC.	• HAW breads: iAUC: 39% < conventional wheat breads (HAW 39 ± 5 mmol/L × 3 h; LAW 64 ± 5 mmol/L × 3 h; $p < 0.0001$). • Insulinemic and incretin: 24–30% less for HAW breads than for LAW breads ($p < 0.05$). • Flour processing did not affect the glycemic, insulinemic, or incretin response. • The HAW breads did not influence plasma ghrelin.	• Replacing LAW with HAW flour may be an effective strategy for lowering postprandial glycemic and insulinemic responses to bread in healthy men and women, but further research is warranted.
Hallström et al. (36)	To evaluate the postprandial glucose and insulin responses *in vivo* to bread products based on a novel wheat genotype with elevated amylose content (EAW) of 38%.	• A randomized cross-over trial. • Healthy 7 females and 7 males (20–35 yrs; BMI: 22.2 ± 1.91) were served test meals on 4 occasions. • RS content (*in vitro*), postprandial glucose and insulin responses.	• Significantly higher RS content in EAW bread than in whole grain wheat bread (WGW) ($p < 0.001$). • EAW induced lower postprandial glucose response than white wheat flour (REF) during the first 120 min ($p < 0.05$), but no significant differences in insulin responses. • Increased RS content per test portion was correlated to a reduced GI ($r = -0.571$, $p < 0.001$).	• Wheat with EAW may be preferable to other wheat genotypes considering RS formation, however further research is required.
Poquette et al. (37)	To measure the contents of functional starch fractions, SDS and RS, and to investigate the effects of grain sorghum on postprandial plasma glucose and insulin levels.	• A randomized-crossover design. • 10 healthy males (25.1 ± 4 yrs) consumed grain sorghum and whole wheat flour (control) muffins containing 50 g total starch with a 1 wk washout period. • Measurement of glucose and insulin levels at 15 min before and 0, 15, 30, 45, 60, 75, 90, 120, 180 min after consumption.	• Mean glucose and insulin responses reduced at 45–120 min and 15†90 min with grain sorghum, compared to control ($p < 0.05$). • The iAUC was significantly lowered for plasma glucose responses ($p < 0.05$). • Significant reduction with insulin responses with sorghum ($p < 0.05$).	• Grain sorghum is a good functional ingredient to assist in managing glucose and insulin levels in healthy individuals.
Gu (38)	To investigate the effects of sorghum starch on postprandial blood glucose and insulin levels in pre-diabetic men	• Grain sorghum and wheat (control) muffins containing 50 g total starch were consumed by 15 pre-diabetic males on two mornings with a 1 wk washout period. • Measurement of glucose and insulin levels at −15 (baseline), 0, 15, 30, 45, 60, 75, 90, 120, and 180 min after each treatment.	• The functional starch content [combined SDS and RS] of grain sorghum muffin was higher than control. • Postprandial blood glucose and insulin responses were both significantly reduced at 45–120 min intervals ($p < 0.05$). • The mean iAUC of glucose and insulin was significantly reduced by 35 and 36.7%, respectively ($p < 0.05$).	• Grain sorghum is a good candidate in controlling blood glucose and insulin levels in pre-diabetic population for the prevention of T2DM.
Lin et al. (39)	To evaluate the effects of the new RS formula, PPB-R-203, on glucose homeostasis in healthy subjects and subjects with T2DM.	• A cohort consisting of 40 healthy participants (20–65 yrs) received test and control diets. • A randomized, 2-regimen, cross-over, comparative study was conducted in 44 subjects (20–65 yrs) with T2DM and glycemic control was assessed with a continuous glucose monitoring system. • Determination of blood glucose and iAUC	• Serum glucose values and iAUC were significantly lower in the PPB-R-203 than the control group, for healthy subjects ($p < 0.05$). • In patients with T2DM, mean blood glucose concentrations for control regimen were higher than the PPB-R-203-based regimen ($p = 0.023$). • AUCs for total blood glucose and hyperglycemia were also reduced for subjects on the PPB-R-203- compared to control (total blood glucose: $p < 0.001$; hyperglycemia: $p = 0.021$).	• A PPB-R-203-based diet reduced postprandial hyperglycemia in patients with T2DM without increasing the risk of hypoglycemia or glucose excursion.
Sanders et al. (40)	To evaluate the effect of consuming cooked, then chilled potatoes, compared to isoenergetic, CHO-containing control foods.	• A pilot cross-over randomized controlled trial. • 19 adults (18–74 yrs;BMI 27.0–39.9)consumed 300 g/day RS-enriched potatoes, over a 24 h period. • Assessment of insulin sensitivity, fasting plasma glucose and fasting insulin	• No significant difference for insulin sensitivity between potato and control. • Lower fasting plasma glucose ($p = 0.043$) with potato compared to control. • Lower fasting insulin ($p = 0.077$) in the potato vs. control.	• RS-enriched potatoes may have a favorable impact on CHO metabolism and support the view that additional research in a larger study sample is warranted.

(Continued)

TABLE 1 | Continued

References	Objective (s)	Methods	Findings	Conclusion
Mohan et al. (41)	To compare the GI of a newly developed high fiber white rice (HFWR) with that of commercial white rice (WR)	• A randomized controlled crossover study design. • 30 healthy adults age 18–45 yrs were recruited for the GI study of HFWR in 2013 • In 2014, GI testing of the second harvest HFWR was done in a subsample of 15 healthy volunteers. • HFWR and WR diets providing 50 g of available CHO (63.6 g of uncooked rice) were given as test foods	• Dietary fiber content of HFWR was 5-fold higher. • RS content of HFWR was 6.5-fold higher ($p < 0.001$) • Amylose content of HFWR was significantly higher ($p < 0.001$) compared with WR • HFWR had 23% lower GI compared with WR ($p = 0.002$).	• HFWR has lower GI excellent sensory and other characteristics compared with WR. • Switching from the current high GI WR to HFWR could help to reduce overall dietary GI and the glycemic load.
Nomura et al. (42)	To evaluate the postprandial glycemic response for boiled BARLEYmax® and determined its GI in a Japanese population.	• 11 healthy subjects (20–50 yrs) were administered a 50 g/150 mL glucose drink twice and boiled BARLEYmax® containing 50 g available CHO after a wash-out period. • Determination of blood glucose, postprandial blood glucose, glucose iAUC and GI of BARLEYmax®.	• Postprandial blood glucose, its change from baseline over 90 min, and the iAUC for BARLEYmax® were statistically lower than those for the glucose drink. • The GI of the BARLEYmax® was 24.3.	• Boiled BARLEYmax® contributes to improving the postprandial glycemic response.
Zhu et al. (43)	To examine the possibility of integrating domestically cooked non-cereal starchy foods into glycemic management diet, and compare their glycemic characteristics with those of waxy and non-waxy whole grains and starchy beans.	• An undouble-blind, randomized crossover design. • 10 healthy subjects (18–26 yrs) consumed dried lily bulb (LB), lotus seed (LS), adlay (AD), waxy black rice (BR), millet (MI), and adzuki bean (AB), pre-soaked. • *In vitro* CHO digestion for each test food.	• Both the LS and AB meals achieved low GI (21–51), while the other starchy foods failed to show significant difference with rice GI (83–109). • The hydrolysis indexes of LS and AB were 37.7–61.1%, significantly lower than other test foods. • The *in vitro* tests indicated that pre-soaking resulted in high RDS and low RS.	• Careful choice of whole grain materials, minimized pre-soaking, and moderate cooking may be critical factors for successful postprandial glycemic management for diabetic and pre-diabetic.
Yulianto et al. (44)	To evaluate content of RS, and GI of Cr—fortified—parboiled rice (Cr-PR) coated with herbal extracts.	• 18 non-diabetic volunteers were recruited to test on GI of the cooked Cr-PR coated with herbal extract. • Unhulled rice and forticant used (Ciherang and CrCl3). Three herbal extracts used were cinnamon bark powder, pandan leaf and bay leaf. • Determination of RS content by enzymatic process.	• RS content of Cr-PR coated with herbal extracts ranged between 8.27 and 8.84%. • Cr-PR coated with herbal extract of 3% had higher RS levels than herbal extracts of 6% and 9% ($p < 0.05$). • Rice coated with 3% cinnamon extract showed the highest RS content (8.84%). • The lowest GI (29–30) was attained by the Cr-PR coated with cinnamon extract of 6–9%.	• The low GI of Cr-PR may be more influenced by the potential of polyphenolic compounds in the herbal extract than its RS levels.
Sandberg et al. (45)	To investigate the effect of WG rye-based products on glucose- and appetite regulation.	• A crossover overnight study design. • 21 healthy subjects (25.3 ± 3.9 yrs) were provided four rye-based evening test meals of either WG rye flour bread (RFB) or a 1:1 ratio of WG rye flour and rye kernels bread (RFB/RKB), with or without added RS. • Determination of blood glucose, insulin, peptide YY (PYY), FFA, IL-6, *ad libitum* energy intake as well as breath H_2 and subjective rating of appetite.	• The evening meal with RFB/RKB + RS decreased postprandial glucose- and iAUC ($p < 0.05$). • All rye-based evening meals decreased or tended to decrease fasting FFA ($p < 0.05$, RFB/RKB: $p = 0.057$). • The evening meal comprising RFB/RKB + RS resulted in an increased p-PYY concentration at fasting (+17%, $p < 0.05$). • No effects on energy intake or IL-6 compared to WWB. • All rye-based evening meals resulted in increased breath H_2 levels at fasting, that remained increased after the standardized breakfast ($p < 0.001$).	• WG rye bread has the potential to improve cardiometabolic variables in an 11–14.5 h perspective in healthy humans. • The combination RFB/RKB + RS positively affected biomarkers of glucose- and appetite regulation in a semi-acute perspective. Meanwhile, RFB and RFB/RKB improved subjective appetite ratings.

(Continued)

TABLE 1 | Continued

References	Objective (s)	Methods	Findings	Conclusion
Sandberg et al. (46)	To investigate the effects of short-term intervention with WG rye on cognitive functions, mood and cardiometabolic risk markers in MID test subjects.	• Crossover study • 38 healthy MID subjects consumed rye-based breads made up of WG rye kernel/flour (1:1) supplemented with RS2 (RB + RS2) for three consecutive days, with white wheat flour bread as reference. • Cognitive function, mood and cardiometabolic risk markers were determined the following morning, 11 – 14 h post intake.	• RB + RS2 increased insulin sensitivity ($p < 0.05$), (PYY, $p < 0.05$; GLP-2, $p < 0.01$) and fasting concentrations of plasma acetate, butyrate and total SCFA ($p < 0.001$). • Fasting levels of IL – 1β were decreased ($p < 0.05$). • No significant difference for other inflammatory markers (CRP, IL-6, IL-18 and LBP) and blood lipids (FFA and TG). • Insulin sensitivity was positively correlated with working memory test performance ($p < 0.05$).	• This study displays novel findings regarding effects of WG rye products on mood, and glucose and appetite regulation in MID subjects, indicating anti-diabetic properties of WG rye. • The beneficial effects are suggested to be mediated through gut fermentation of dietary fiber in the RB + RS2 product.
Nichenametla et al. (47)	To examine the effects of a blinded exchange of RS4-enriched flour (30% v/v) with regular/control flour (CF) diet on multiple metabolic syndrome (MetS) comorbidities.	• A double blind, placebo-controlled, cluster cross-over intervention. • 86 male and female subjects (≥ 18 yrs) consumed RS4-enriched flour (30% v/v) and regular flour as control with 2 wks washout period • Determination of glucose profile (FPG, post-prandial glucose, and HbA1C).	• RS4 consumption resulted in 7.2% ($p = 0.002$) lower mean TC, 5.5% ($p = 0.04$) lower non-HDL, and a 12.8% ($p < 0.001$) lower HDL in the MetS group. • No significant effect of RS4 was observed for glycemic variables (FPG, postprandial glucose, and HbA1C) and blood pressures.	• RS4 consumption improved dyslipidemia and body composition.
García et al. (48)	To evaluate the glycemic control and cardiovascular risk biomarkers in fragile, ELD T2DM patients after the intake of a new fructose-free diabetes-specific formula enriched with RS4 and high in monounsaturated FAs.	• An experimental, prospective, intention-to-treat clinical trial. • 41 patients with T2DM (78.9 ± 2.8 yrs) were fed exclusively with an enteral diabetes-specific formula for 6 wks. • Data were collected at baseline and after 6 wks of feeding. • CHO and lipid metabolism, inflammatory and cardiovascular risk biomarkers were measured.	• Blood HbA1c significantly decreased after the intervention ($p < 0.05$), as well as monocyte chemotactic protein-1 (MCP-1) and soluble E-selectin ($p < 0.05$). • Soluble vascular cell adhesion molecule-1 (sVCAM-1) and plasminogen activator inhibitor-1 (PAI-1) tended to decrease from baseline to 6 wks ($p = 0.084$ and $p = 0.05$, respectively).	• The new product improves glycemic control and cardiovascular risk without altering lipid metabolism, which is useful for the prevention of diabetic complications. • Longer intervention studies are needed.
Rahat-Rozenbloom et al. (49)	To compare the effects of two fermentable fibers on postprandial SCFA and second-meal glycemic response in healthy overweight or obese (OWO) vs. lean (LN) participants.	• Randomized crossover design. • Male and non-pregnant, non-lactating females aged 18–65 yrs. • 13 OWO and 12 LN overnight fasted participants were studied for 6 h on three separate days after consuming 300 mL water containing 75 g glucose as control or with 24 g IN or 28 g RS. • Determination of blood and serum glucose, insulin, C-peptide and FFA.	• IN significantly increased serum SCFA ($p < 0.001$) but had no effect on FFA or second-meal glucose and insulin responses compared to control. • RS had no significant effect on SCFA but reduced FFA rebound ($p < 0.001$) and second-meal glucose ($p = 0.002$) and insulin responses ($p = 0.024$). • OWO had similar postprandial serum SCFA and glucose concentrations but significantly greater insulin and FFA than LN. • The effects of IN and RS on SCFA, glucose, insulin and FFA responses were similar in LN and OWO.	• RS has favorable second-meal effects, likely related to changes in FFA rather than SCFA concentrations. However, a longer study may be needed to demonstrate RS effects on SCFA. • No evidence that acute increases in SCFA after IN reduce glycemic responses. • No significant differences detected in SCFA responses between OWO vs. LN subjects.

protection against oxidation which indirectly assists in lowering cardiovascular disease risk among T2DM patients (31). Gargari and co-investigators (32) have conducted a study to determine if RS can react as an alimentary prebiotic for patients with T2DM. They have found that RS2 decreased HbA1c (−0.3%, −3.6%) and TNF-α (−3.4 pg/mL, −18.9%) significantly compared with the placebo group ($p < 0.05$). However, changes in FBS, hs-CRP and IL-6 were not significant in the RS2 group compared with the control group. In contrast to the previous finding by Heianza and co-workers (6), both studies done by Lotfollahi and Gargari recorded decreased HbA1c levels after the intervention (31, 32).

Although RS2 can improve the glycemic status and inflammatory markers in some individuals with T2DM, more studies are needed to confirm the efficacy of RS2 as an adjunct therapy in diabetes, as reported by Gargari et al. (32). Similarly, Alfa and colleagues conducted another study to evaluate the tolerability, glucose, and insulin modulating ability in healthy MID and ELD adults upon consumption of *MSPrebiotic*® digestion resistant starch (DRS) (33). *MSPrebiotic*® is a product made from unmodified natural DRS from potatoes and contains an active ingredient, *Solanum tuberosum* extract. The subjects were randomized and required to consume 30 g/d of either *MSPrebiotic*® or placebo for 12 wks. Several parameters including blood glucose, lipid profile, CRP, TNF-α, IL-10, and insulin resistance (IR) were measured over the whole study period. Baseline data from this study indicated that the ELD population had a significantly higher elevated glucose and TNF-α compared to MID adults. Interestingly, a significant difference was observed in blood glucose, insulin levels and HOMA-IR in ELD subjects who consumed *MSPrebiotic*® compared to placebo. Unfortunately, there were no significant changes found in MID subjects. A newly published systematic review to evaluate the effect of prebiotics on metabolic and inflammatory biomarkers in T2DM patients found that ∼70% of the study reported improvements in glycemia including HBA1c, HOMA-IR and inflammatory markers (64).

Besides its action on diabetic parameters and gut microbiota, RS2 was also found to affect macronutrient oxidation based on a study conducted in healthy adults aged 25–45 yrs in 2019. The subjects who consumed RS and RS high fiber (RSF) demonstrated a 32% increase in fat oxidation ($p < 0.05$) with a concomitant 18% decrease in CHO oxidation ($p < 0.05$). Furthermore, insulin responses were unaltered after breakfast but lower in RS and RSF after lunch, at equivalent glucose concentrations. Based on this finding, RS and RSF were expected to improve insulin sensitivity at later meals, though there has been no earlier expression of insulin response (34). Nevertheless, this study was carried out on healthy adults and dissimilar findings can be documented if the trial was performed on patients with T2DM.

Based on our search, we also noticed that RS3-based interventions had been extensively investigated. In 2019, Belobrajdic and his colleagues (35) had conducted a crossover-controlled study on 20 healthy non-diabetic men who consumed bread made from HAW and LAW. They determined that flour processing did not affect the glycemic, insulinemic or increatin response. However, HAW bread had lower insulinemic and increatin (24–30% less) than LAW (35). In other words, replacing LAW with HAW flour is more practical as an effective strategy for lowering glycemic and insulinemic responses in healthy men. **Figure 3** represents the schematic action of gut nutrients on insulin release (65, 66). From a mechanistic perspective, vast data suggest that acetate has an important regulatory role in body weight control and insulin sensitivity via lipid metabolism and glucose homeostasis.

In another study, healthy subjects who consumed EAW bread showed significantly lower PPG response than those who consumed the reference white wheat bread during the first 120 min ($p < 0.05$), although there were no differences in insulin responses (36). This novel wheat with 38% EAW was field-grown under standard cultural practices in the experimental station of the University of Tuscia (Viterbo, Central Italy). Interestingly, higher RS content in bread significantly reduced the GI ($p < 0.001$). Despite promising results, this study was limited in terms of sample size that does not guarantee effectiveness in a larger population. Hence, further investigations are warranted for a more effective outcome on the roles of EAW in the improvement of postprandial glycemic response (36).

Besides using wheat bread, the effect of grain sorghum flour was also investigated in both healthy and pre-diabetic subjects, respectively (37, 38). Grain sorghum flour contained 22.7 ± 0.8, 43.8 ± 0.8, and 33.5 ± 0.1, whereas wheat flour contained 37.5 ± 0.3, 47.4 ± 0.4, and 15.1 ± 0.1 of RDS, SDS, and RS; respectively. In healthy subjects, incremental changes in plasma glucose and plasma insulin concentrations were recorded with the grain sorghum muffin treatment. Furthermore, the glucose responses were significantly lower, particularly at 45–120 min intervals ($p < 0.05$) and mean insulin responses reduced at 15–90 min intervals compared to control ($p < 0.05$). This study demonstrated a significant reduction at about 26% in the mean glucose iAUC responses with sorghum at 2,871 ± 163 mg compared to wheat at 3,863 ± 443 mg (∼3 h)/dL. Similarly, insulin responses also significantly decreased from 3,029 ± 965 μU (∼3 h)/L for wheat to 1,357 ± 204 with sorghum ($p < 0.05$), respectively. Furthermore, significant reduction on postprandial blood glucose and insulin responses within 45–120 min intervals ($p < 0.05$) along with reduction in glucose iAUC by 35.0%, from 5457.5 ± 645.4 to 3550.0 ± 428.9 mg (∼3 h)/ dL ($p < 0.05$) were recorded in pre-diabetic men. Additionally, the mean insulin iAUC was also significantly lowered by 36.7%, from 7254.6 ± 1228.9 to 4589.3 ± 737.2 mU (∼3 h)/L ($p < 0.05$). In short, both studies documented interesting results that grain sorghum is an excellent functional ingredient to assist in managing glucose and insulin levels among healthy individuals and controlling blood glucose and insulin levels among the pre-diabetic population (37, 38).

A group of researchers has also embarked on a study to evaluate the effect of a new formula, PPB-R-203 (a retrograded starch product manufactured by Pharma Power Biotec Co., Ltd.) vs. commercially available food on blood glucose regulation and safety in both healthy and T2DM individuals aged 20–65 yrs old (39). The findings demonstrated that mean blood

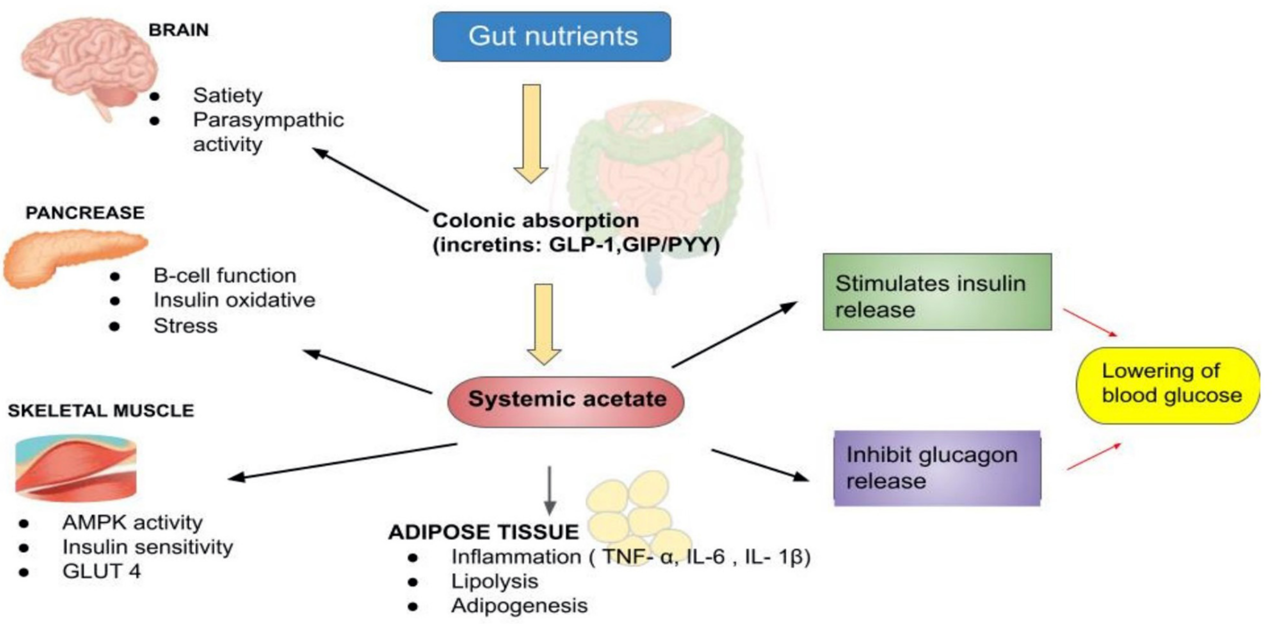

FIGURE 3 | The schematic actions of gut nutrients on insulin release [Adapted from: (65, 66)].

glucose concentration and glucose iAUC were lower with the PPB-R-203 regimen than control among T2DM patients. In brief, PPB-R-203-based food managed to improve postprandial hyperglycemia in T2DM patients without giving a risk of hypoglycemia compared with commercially available food (39).

Recently, a comparison study has been done by Sanders and his friends to assess the metabolic effects in subjects that consumed 300 g of cooked then chilled potatoes containing ∼18 g of RS compared to a control diet matched for calories and macronutrients but with <1 g of RS over a 24 h period (40). By introducing RS-enriched potatoes, the values for muscle insulin sensitivity index (MISI), HOMA2 insulin sensitivity (%S), and HOMA2 β-cell function (%B) increased, although the differences did not approach statistical significance compared with the control group. However, a significant reduction of the FPG ($p < 0.05$) was recorded with a slight insignificant decrease of fasting plasma insulin ($p = 0.077$). In addition, breath hydrogen at the end of 300 min was significantly higher ($p < 0.05$), and plasma FFA was significantly lower ($p < 0.05$) in the potato condition compared to the control. Even though RS-enriched potatoes may have a favorable impact on CHO metabolism, additional research with a large study sample is necessary.

Newly developed high fiber white rice (HFWR) has also been investigated for its GI and effect on glycemic parameters among healthy volunteers. The subjects were given HFWR and commercial white rice (WR) diets containing 50 g of available CHO as the test foods. HFWR was documented with a 23% lower GI value than WR, in which HFWR was found to be of medium GI (61.3) whereas WR was of higher GI (79.2). The dietary fiber, RS, and amylose content were also significantly higher in HFWR than WR. These results showed that the newly developed HFWR had a lower GI and other favorable characteristics than WR. Hence, the HFWR could help reduce the overall dietary GI and the glycemic load (41). However, it may be necessary to conduct an additional study to identify the HFWR capacity on its impact on blood glucose parameters, particularly for those with disabilities in glucose management.

Another valuable product that has been recognized as one of the ancient cultivated cereal grains globally is barley (*Hordeum vulgare* L.). Barley is an enriched source of high fiber contents, at about 15.6 g/100 g (67). Nomura and his colleagues conducted a study to determine the GI, blood glucose, postprandial blood glucose and iAUC after BARLEYmax® consumption in healthy Japanese subjects (42). BARLEYmax® is a unique, non-genetically modified barley developed by Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO) (68). They found that the GI of the BARLEYmax® was 24.3, and glucose iAUC was statistically lower than those who took the ordinary glucose drink. They presumed that the presence of fructans in BARLEYmax® at 11% could contribute to glycemic response reduction, which is higher than other components comprised of RS, β-glucan and arabinoxylan at 3, 6 and 7%, respectively (42).

Meanwhile, Zhu and his colleagues have investigated the potential benefits of a combination of several non-cereal starchy (LS, AD and dried LB) compared with (MI, BR and AB) into a glycemic management diet while taking the pre-soaking and cooking duration into account (43). Unfortunately, despite being whole grains, the waxy and pre-soaked cereals can be very high-GI food. Moreover, pulses such as the AB can maintain a low GI even after a long-time pre-soaking and prolonged cooking and the LS is a valuable low-GI food ingredient in staple food which behaved more like pulses in terms of GR, while the AD and LB were more like millet and strongly dependent on cooking practice for GI values. Thus, they concluded that careful choice of whole-grain materials, minimized pre-soaking, and moderate cooking may be critical for successful glycemic management for people with impaired glucose metabolism. On the contrary, Yulianto and his friends evaluated the content of RS and GI of Cr-PR coated with herbal extracts (cinnamon bark powder, pandan leaf and bay leaf) with concentrations of Cr-PR at 1, 2, and 3%, respectively (44). In their study, 18 non-diabetic individuals were required to consume one type of cooked rice sample equivalent to 50 g of glucose. They recorded that the RS content of Cr-PR coated with herbal extracts ranged between 8.27 and 8.84% and the highest RS was gained from coating rice with 3% cinnamon extract. On the whole, it was classified into the low GI category as it ranged from 29 to 40 and the lowest GI was observed in Cr-PR coated with 6–9% of cinnamon extract (29–30) (44).

So far, no specific high-fiber or CHO-rich foods are arguably superior to the others. Sandberg and his colleagues have conducted a randomized, controlled, crossover overnight study to investigate the effect of WG-based products on glucose and appetite regulation (45). In this noteworthy study, four rye-based evening test meals were given to 21 healthy subjects consisting of either whole-grain RFB or a 1:1 ratio of RFB/RKB, with or without added RS, while WWB was used as the reference meal. Interestingly, the findings showed that the evening test meal of RFB/RKB + RS resulted in decreased postprandial glucose and insulin responses and increased the gut hormone in plasma the following morning after the standardized breakfast. Apart from that, consumption of RFB and RFB/RKB also resulted in a decreased feeling of hunger in the test subjects (AUC 0–210 min), while all the rye-based evening meals resulted in reduced fasting FFA and increased breath hydrogen concentration. This finding suggests that WG bread had the potential to improve cardiometabolic variables at 11 to 14.5 h perspective in healthy humans (45).

Research on cardiometabolic risk markers that consumed RB has also been evaluated in 38 healthy individuals aged 52–70 yrs. Insulin sensitivity index, PYY levels and plasma acetate fasting concentrations, butyrate, and total SCFA were recorded to increase with RB + RS2 bread product consumption. In addition, the insulin sensitivity index was found to correlate positively with working memory test performance (**Figure 3**). Intriguingly, IL−1β fasting levels were found to decrease after the intervention (46).

Besides RS2- and RS3-based diets studied by numerous researchers, wheat-based RS4-enriched flour (30% v/v) has also been investigated in 86 subjects above 18 yrs old (47). Except for TG, the RS4-enriched flour significantly reduced cholesterol levels (TC, HDL and non-HDL, including LDL). Although mean blood glucose levels were lowered with RS4 consumption, the differences did not approach statistical significance compared to the control. Mesa Garcia and her colleagues have taken a step ahead of research to study a new fructose-free DSF in fragile elderly T2DM patients for 6 wks and evaluate the influence of total enteral feeding on the glycemic control and diabetes-derived cardiovascular risk biomarkers (48). These new fructose-free DSF and RS4-enriched DSF formulations effectively controlled glycemic by decreasing blood HbA1c, and some improvements were documented in MCP-1, sE-selectin, and sVCAM-1 and PAI-1 plasma levels. While this formulation managed to improve glycemic control and cardiovascular risk biomarkers, lipid metabolism has not changed, which is helpful for the nutritional treatment of frail elderly T2DM patients.

Apart from RS4- enriched flour and DSF formulations, two fermentable-fibers were also investigated on postprandial SCFA and second-meal glycemic response among healthy OWO vs. LN participants (NCT02562014) (49). Intriguingly, no significant differences were observed in postprandial SCFA responses between LN and OWO participants after acute fiber consumption. They reported that any effect of the colonic fermentation of dietary fiber on glycemic responses was not attributed to SCFA, as IN increased SCFA serum without reducing the second-meal glycemic response. The low glucose response after RS consumption at 4–6 h is consistent with other studies that find the RS therapy increases insulin susceptibility and reduces insulin and C-peptide responses (49). The decreased glycemic reaction is probably not related to SCFA but may be related to the decrease in FFA recovery. A higher-than-expected amount of available but SDS in the RS ingredient used would have explained the acute reduction of FFA, as both prolonged and enhanced absorption of CHO managed to reduce postprandial FFA rebound. Based on these findings, they predicted that IN is fermented promptly in the colon and that breath-hydrogen and serum SCFA concentrations are rapidly rising, while RS is fermented gradually for 6 h after consumption with far smaller increases of breath hydrogen and serum SCFA. Moreover, the results did not demonstrate the impact on glucose metabolism of the acute increments in SCFA. In addition, the results did not support the hypothesis that colonial SCFA was increased due to obesity/overweight conditions (49).

CONCLUSION

In conclusion, our review highlighted the benefits of RS that are loaded with antidiabetic properties. A considerable amount of RS consumption acts differently through several pathways to reduce the severity and exert better control of the diabetic condition. Although a specific RS type could not be highlighted for a better action mechanism, we observed that both RS2 and RS3 were among the most extensively explored form of RS over the last decade. The search results demonstrated that most of the studies were focused on insulin secretion and plasma glucose. Nevertheless, the RS effectiveness on HbA1c, inflammatory markers and lipid profile were also investigated in several studies. However, a proper comparison and conclusion are deemed inappropriate in view that the study parameters varied in terms of study duration, sample size, subjects and their metabolic conditions, intervention doses, and the intervention base products. Furthermore, the underlying mechanisms involved in the action of RS for diabetes control should be explored in more detailed perspectives for a better understanding.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

1. Goldenberg R, Punthakee Z. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diab*. (2013) 37:S8–11. doi: 10.1016/j.jcjd.2013.01.011
2. Colagiuri S, Lee CM, Wong TY, Balkau B, Shaw JE, Borch-Johnsen K, et al. Glycemic thresholds for diabetes-specific retinopathy: implications for diagnostic criteria for diabetes. *Diab Care*. (2011) 34:145–50. doi: 10.2337/dc10-1206
3. Ito C. Evidence for diabetes mellitus criteria in 2010 using HbA1c. *Diabetol Int*. (2013) 4:9–15. doi: 10.1007/s13340-012-0086-7
4. Kowall B, Rathmann W. HbA1c for diagnosis of type 2 diabetes. Is there an optimal cut point to assess high risk of diabetes complications, and how well does the 6.5% cutoff perform? *Diab Metab Syndrome Obes.* (2013) 6:477. doi: 10.2147/DMSO.S39093
5. Huang Y, Cai X, Mai W, Li M, Hu Y. Association between prediabetes and risk of cardiovascular disease and all cause mortality: systematic review and meta-analysis. *BMJ*. (2016) 355. doi: 10.1136/bmj.i5953
6. Heianza Y, Arase Y, Fujihara K, Tsuji H, Saito K, Hsieh S, et al. Screening for pre-diabetes to predict future diabetes using various cut-off points for HbA1c and impaired fasting glucose: the Toranomon Hospital Health Management Center Study 4 (TOPICS 4). *Diab Med.* (2012) 29:e279–85. doi: 10.1111/j.1464-5491.2012.03686.x
7. Association AD. Diagnosis and classification of diabetes mellitus. *Diab Care*. (2013) 36(Suppl. 1):S67. doi: 10.2337/dc13-S067
8. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes–global burden of disease and forecasted trends. *J Epidemiol Glob Health*. (2020) 10:107. doi: 10.2991/jegh.k.191028.001
9. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, et al. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Sci Rep.* (2020) 10:1–11. doi: 10.1038/s41598-020-71908-9
10. WHO. *Diet, Nutrition and Prevention of Chronic Diseases*. Geneva: World Health Organ Tech Rep Ser (2002).
11. Gentile CL, Ward E, Holst JJ, Astrup A, Ormsbee MJ, Connelly S, et al. Resistant starch and protein intake enhances fat oxidation and feelings of fullness in lean and overweight/obese women. *Nutr J.* (2015) 14:1–10. doi: 10.1186/s12937-015-0104-2
12. Priebe M, van Binsbergen J, de Vos R, Vonk RJ. Whole grain foods for the prevention of type 2 diabetes mellitus. *Cochrane Database Syst Rev.* (2008) CD006061. doi: 10.1002/14651858.CD006061.pub2
13. Meenu M, Xu B. A critical review on anti-diabetic and anti-obesity effects of dietary resistant starch. *Crit Rev Food Sci Nutr.* (2019) 59:3019–31. doi: 10.1080/10408398.2018.1481360
14. Englyst HN, Kingman SM, Cummings J. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr.* (1992) 46:S33–50.
15. Tharanathan RN. Food-derived carbohydrates—structural complexity and functional diversity. *Crit Rev Biotechnol.* (2002) 22:65–84. doi: 10.1080/07388550290789469
16. Sánchez-Zapata E, Viuda-Martos M, Fernández-López J, Pérez-Alvarez J. Resistant starch as functional ingredient. *Polysacch Bioactivity Biotechnol.* (2015) 43:1911–31. doi: 10.1007/978-3-319-16298-0_34
17. Information NCfB. *PubChem Compound Summary for CID 53477911, 1,4-beta-D-Glucan*. (2021). Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/1_4-beta-D-Glucan (accessed June 4, 2021).
18. Mikulíková D, Masár Š, Kraic J. Biodiversity of legume health-promoting starch. *Starch Stärke*. (2008) 60:426–432. doi: 10.1002/star.200700693
19. Gao C, Rao M, Huang W, Wan Q, Yan P, Long Y, et al. Resistant starch ameliorated insulin resistant in patients of type 2 diabetes with obesity: a systematic review and meta-analysis. *Lipids Health Dis*. (2019) 18:205. doi: 10.1186/s12944-019-1127-z
20. Maziarz MP. Role of fructans and resistant starch in diabetes care. *Diab Spectrum*. (2013) 26:35–9. doi: 10.2337/diaspect.26.1.35
21. Nugent AP. Health properties of resistant starch. *Nutr Bull.* (2005) 30:27–54. doi: 10.1111/j.1467-3010.2005.00481.x
22. Hasjim J, Ai Y, Jane J-l. Novel applications of amylose-lipid complex as resistant starch type 5. In: Shi Y-C, Maningat CC. *Resistant Starch*. (2013) 79–94. doi: 10.1002/9781118528723.ch04
23. Perez-Alvarez J, López J. Overview of meat products as functional foods. *Technol Strat Funct Meat Prod Dev*. (2008) 1–17.
24. Carlson JJ, Eisenmann JC, Norman GJ, Ortiz KA, Young PC. Dietary fiber and nutrient density are inversely associated with the metabolic syndrome in US adolescents. *J Am Dietetic Assoc.* (2011) 111:1688–95. doi: 10.1016/j.jada.2011.08.008
25. Heijnen M, Van Amelsvoort J, Deurenberg P, Beynen AC. Limited effect of consumption of uncooked (RS2) or retrograded (RS3) resistant starch on putative risk factors for colon cancer in healthy men. *Am J Clin Nutr.* (1998) 67:322–31. doi: 10.1093/ajcn/67.2.322
26. Bodinham CL, Smith L, Wright J, Frost GS, Robertson MD. Dietary fibre improves first-phase insulin secretion in overweight individuals. *PLoS ONE*. (2012) 7:e40834. doi: 10.1371/journal.pone.0040834
27. Bodinham C, Smith L, Thomas EL, Bell JD, Swann JR, Costabile A, et al. Efficacy of increased resistant starch consumption in human type 2 diabetes. *Endocr Connect.* (2014) 3:75–84. doi: 10.1530/EC-14-0036
28. Dainty SA, Klingel SL, Pilkey SE, McDonald E, McKeown B, Emes MJ, et al. Resistant starch bagels reduce fasting and postprandial insulin in adults at risk of type 2 diabetes. *J Nutr.* (2016) 146:2252–9. doi: 10.3945/jn.116.239418
29. Peterson CM, Beyl RA, Marlatt KL, Martin CK, Aryana KJ, Marco ML, et al. Effect of 12 wk of resistant starch supplementation on cardiometabolic risk factors in adults with prediabetes: a randomized controlled trial. *Am J Clin Nutr*. (2018) 108:492–501. doi: 10.1093/ajcn/nqy121
30. Kwak JH, Paik JK, Kim HI, Kim OY, Shin DY, Kim H-J, et al. Dietary treatment with rice containing resistant starch improves markers of endothelial function with reduction of postprandial blood glucose and oxidative stress in patients with prediabetes or newly diagnosed type 2 diabetes. *Atherosclerosis*. (2012) 224:457–64. doi: 10.1016/j.atherosclerosis.2012.08.003
31. Lotfollahi Z, Mello APQ, Costa ES, Oliveira CLP, Damasceno NRT, Izar MC, et al. Green-banana biomass consumption by diabetic patients improves plasma low-density lipoprotein particle functionality. *Sci Rep*. (2020) 10:12269. doi: 10.1038/s41598-020-69288-1
32. Gargari BP, Namazi N, Khalili M, Sarmadi B, Jafarabadi MA, Dehghan P. Is there any place for resistant starch, as alimentary prebiotic, for patients with type 2 diabetes? *Compl Ther Med.* (2015) 23:810–5. doi: 10.1016/j.ctim.2015.09.005
33. Alfa MJ, Strang D, Tappia PS, Olson N, DeGagne P, Bray D, et al. A randomized placebo controlled clinical trial to determine the impact of digestion resistant starch MSPrebiotic® on glucose, insulin, and insulin resistance in elderly and mid-age adults. *Front Med.* (2018) 4:260. doi: 10.3389/fmed.2017.00260
34. Giles ED, Brown IL, MacLean PS, Pan Z, Melanson EL, Heard KJ, et al. The *in vivo* net energy content of resistant starch and its effect on macronutrient oxidation in healthy adults. *Nutrients*. (2019) 11:2484. doi: 10.3390/nu11102484

35. Belobrajdic DP, Regina A, Klingner B, Zajac I, Chapron S, Berbezy P, et al. High-amylose wheat lowers the postprandial glycemic response to bread in healthy adults: a randomized controlled crossover trial. *J Nutr.* (2019) 149:1335–45. doi: 10.1093/jn/nxz067
36. Hallström E, Sestili F, Lafiandra D, Björck I, Östman E. A novel wheat variety with elevated content of amylose increases resistant starch formation and may beneficially influence glycaemia in healthy subjects. *Food Nutr Res.* (2011) 55:7074. doi: 10.3402/fnr.v55i0.7074
37. Poquette NM, Gu X, Lee S-O. Grain sorghum muffin reduces glucose and insulin responses in men. *Food Funct.* (2014) 5:894–899. doi: 10.1039/C3FO60432B
38. Gu X. *Effects of Grain Sorghum Muffin on Blood Glucose and Insulin Responses in Prediabetic Men*. Fayetteville: University of Arkansas (2014).
39. Lin C-H, Chang D-M, Wu D-J, Peng H-Y, Chuang L-M. Assessment of blood glucose regulation and safety of resistant starch formula-based diet in healthy normal and subjects with type 2 diabetes. *Medicine*. (2015) 94:e1332. doi: 10.1097/MD.0000000000001332
40. Sanders L, Dicklin M, Palacios O, Maki C, Wilcox M, Maki K. Effects of potato resistant starch intake on insulin sensitivity, related metabolic markers and appetite ratings in men and women at risk for type 2 diabetes: a pilot cross-over randomised controlled trial. *J Hum Nutr Dietetics*. (2021) 34:94–105. doi: 10.1111/jhn.12822
41. Mohan V, Anjana RM, Gayathri R, Ramya Bai M, Lakshmipriya N, Ruchi V, et al. Glycemic index of a novel high-fiber white rice variety developed in India—A randomized control trial study. *Diab Technol Ther*. (2016) 18:164–70. doi: 10.1089/dia.2015.0313
42. Nomura N, Miyoshi T, Hamada Y, Kitazono E. Glycemic index of boiled BARLEYmax® in healthy Japanese subjects. *J Cereal Sci.* (2020) 93:102959. doi: 10.1016/j.jcs.2020.102959
43. Zhu R, Fan Z, Han Y, Li S, Li G, Wang L, et al. Acute effects of three cooked non-cereal starchy foods on postprandial glycemic responses and *in vitro* carbohydrate digestion in comparison with whole grains: a randomized trial. *Nutrients*. (2019) 11:634. doi: 10.3390/nu11030634
44. Yulianto W, Suryani C, Susiati A, Luwihana S. Evaluation of chromium fortified-parboiled rice coated with herbal extracts: resistant starch and glycemic index. *Int Food Res J.* (2018) 25:2608–13.
45. Sandberg JC, Björck IM, Nilsson AC. Effects of whole grain rye, with and without resistant starch type 2 supplementation, on glucose tolerance, gut hormones, inflammation and appetite regulation in an 11–14.5 hour perspective; a randomized controlled study in healthy subjects. *Nutr J.* (2017) 16:1–11. doi: 10.1186/s12937-017-0246-5
46. Sandberg JC, Björck IME, Nilsson AC. Impact of rye-based evening meals on cognitive functions, mood and cardiometabolic risk factors: a randomized controlled study in healthy middle-aged subjects. *Nutr J.* (2018) 17:102. doi: 10.1186/s12937-018-0412-4
47. Nichenametla SN, Weidauer LA, Wey HE, Beare TM, Specker BL, Dey M. Resistant starch type 4-enriched diet lowered blood cholesterols and improved body composition in a double blind controlled cross-over intervention. *Mol Nutr Food Res.* (2014) 58:1365–9. doi: 10.1002/mnfr.201300829
48. García MDM, García-Rodríguez CE, de la Cruz Rico M, Aguilera CM, Pérez-Rodríguez M, Pérez-de-la-Cruz AJ, et al. A new fructose-free, resistant-starch type IV-enriched enteral formula improves glycaemic control and cardiovascular risk biomarkers when administered for six weeks to elderly diabetic patients. *Nutricion Hospital.* (2017) 34:73–80. doi: 10.20960/nh.978
49. Rahat-Rozenbloom S, Fernandes J, Cheng J, Gloor GB, Wolever TM. The acute effects of inulin and resistant starch on postprandial serum short-chain fatty acids and second-meal glycemic response in lean and overweight humans. *Eur J Clin Nutr.* (2017) 71:227–33. doi: 10.1038/ejcn.2016.248
50. Murphy MM, Douglass JS, Birkett A. Resistant starch intakes in the United States. *J= Am Dietetic Assoc.* (2008) 108:67–78. doi: 10.1016/j.jada.2007.10.012
51. Wahren J, Ekberg K, Johansson J, Henriksson M, Pramanik A, Johansson B-L, et al. Role of C-peptide in human physiology. *Am J Physiol Endocrinol Metab.* (2000) 278:E759–68. doi: 10.1152/ajpendo.2000.278.5.E759
52. Kahn SE, Porte D, Insulin secretion in the normal and diabetic human. In: Alberti KGMM ZP, Defronzo RA, Keen H, editor. *International Textbook of Diabetes Mellitus*. New York, NY: John Wiley & Sons (1997). p. 337–54.
53. Association AD. Postprandial blood glucose. *Diabetes Care*. (2001) 24:775–8. doi: 10.2337/diacare.24.4.775
54. Woerle HJ, Neumann C, Zschau S, Tenner S, Irsigler A, Schirra J, et al. Impact of fasting and postprandial glycemia on overall glycemic control in type 2 diabetes: importance of postprandial glycemia to achieve target HbA1c levels. *Diab Res Clin Pract.* (2007) 77:280–5. doi: 10.1016/j.diabres.2006.11.011
55. Schirra J, Wank U, Arnold R, Göke B, Katschinski M. Effects of glucagon-like peptide-1 (7–36) amide on motility and sensation of the proximal stomach in humans. *Gut*. (2002) 50:341–8. doi: 10.1136/gut.50.3.341
56. Meier JJ, Gallwitz B, Salmen S, Goetze O, Holst JJ, Schmidt WE, et al. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab.* (2003) 88:2719–25. doi: 10.1210/jc.2003-030049
57. Shah M, Vella A. Effects of GLP-1 on appetite and weight. *Rev Endocr Metab Disord.* (2014) 15:181–7. doi: 10.1007/s11154-014-9289-5
58. Canada PHAo. *The Canadian Diabetes Risk Questionnaire*. Government of Canada (2011).
59. Galarregui C, Navas-Carretero S, Gonzalez-Navarro CJ, Martinez JA, Zulet MA, Abete I. Both macronutrient food composition and fasting insulin resistance affect postprandial glycemic responses in senior subjects. *Food Funct.* (2021) 12:6540–8. doi: 10.1039/D1FO00731A
60. Zhao H, Huang X, Jiao J, Zhang H, Liu J, Qin W, et al. Protein phosphatase 4 (PP4) functions as a critical regulator in tumor necrosis factor (TNF)-α-induced hepatic insulin resistance. *Sci Rep.* (2015) 5:18093. doi: 10.1038/srep18093
61. Correia ML, Haynes WG. A role for plasminogen activator inhibitor-1 in obesity: from pie to PAI? *Arterioscler Thromb Vasc Biol.* (2006) 26:2183–5. doi: 10.1161/01.ATV.0000244018.24120.70
62. Palau-Rodriguez M, Tulipani S, Isabel Queipo-Ortuño M, Urpi-Sarda M, Tinahones FJ, Andres-Lacueva C. Metabolomic insights into the intricate gut microbial–host interaction in the development of obesity and type 2 diabetes. *Front Microbiol.* (2015) 6:1151. doi: 10.3389/fmicb.2015.01151
63. Sami W, Ansari T, Butt NS, Ab Hamid MR. Effect of diet on type 2 diabetes mellitus: a review. *Int J Health Sci.* (2017) 11:65.
64. Colantonio AG, Werner SL, Brown M. The effects of prebiotics and substances with prebiotic properties on metabolic and inflammatory biomarkers in individuals with type 2 diabetes mellitus: a systematic review. *J Acad Nutr Dietetics*. (2020) 120:587–607.e2. doi: 10.1016/j.jand.2018.12.013
65. Holst JJ, Deacon CF. Is there a place for incretin therapies in obesity and prediabetes? *Trends Endocrinol Metab.* (2013) 24:145–52. doi: 10.1016/j.tem.2013.01.004
66. González Hernández MA, Canfora EE, Jocken JW, Blaak EE. The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients*. (2019) 11:1943. doi: 10.3390/nu11081943
67. Azam A, Itrat N, Ahmed U. Hypoglycemic effect of barley (*Hordeum vulgare*) in diabetics. *Group.* (2019) 37:165.8–3.67.
68. Morell MK, Kosar-Hashemi B, Cmiel M, Samuel MS, Chandler P, Rahman S, et al. Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant J.* (2003) 34:173–85. doi: 10.1046/j.1365-313X.2003.01712.x

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Enrichment in Different Health Components of Barley Flour Using Twin-Screw Extrusion Technology to Support Nutritionally Balanced Diets

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Due to its good dietary role, barley has attracted a growing amount of interest for the manufacture of functional foods in recent years. In barley, a number of bioactive components, including as phenolic compounds, have been discovered, and barley extrudates could be used to formulate various processed foods, including ready-to-eat cereals, baby, and pet foods and support nutritionally balanced diets. This study was conducted to investigate the effect of extrusion processing on resistant starch (RS), glycemic index (GI), and antioxidant compounds of barley flour. The L^* and ΔE values of barley flours decreased significantly ($p < 0.05$) after extrusion is done at 150 and 180°C. The a^* and b^* values, however, increased after extrusion. Extrusion increased antioxidant activity (AOA), metal chelating activity (MCA), and $ABTS^+$ scavenging activity, whereas total phenolic content (TPC) and total flavonoids content (TFC) decreased. Barley extrudates at 150 and 180°C showed decreased TPC by 16.4–34.2% and 23.4–38.1%. Moreover, improved RS and reduced GI values were recorded for barley extrudates as compared to barley non-extrudates. Therefore, extrusion of barley could be an alternative to produce pregelatinized barley flour with improved RS low GI values and improved antioxidant potential.

Keywords: barley, extrusion, resistant starch, phenolic, antioxidants

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a grass family member and an ancient functional cereal crop grown in temperate climates worldwide. Globally, almost 51 million hectares land is cultivated for barley with a total production of 159 million tons (1). According to current knowledge, barley is the oldest cultivated grain in human history. It is mainly used for brewing beer. Barley mucilage has a positive effect on stomach diseases. Barley flour is not suitable for baking, as the bread tends to crack and crumble due to the fact it is not as rich in gluten as other grains. Gluten ensures that the bread rises in the baking process and keeps its shape.

Unlike in the past, most barley is currently processed into animal feed, it can be proven that the oldest cultivated cereal was also important for human nutrition in the past (2). Bread and baked goods could not be made from barley, as the fiber-rich grain has no baking properties, but it could be used to make a satisfying porridge. The advantage of barley bread, according to researchers, is its long shelf life and high fiber content. The researchers are particularly interested in the so-called beta-glucans, a soluble dietary fiber with cholesterol-regulating and blood sugar-lowering effects (3).

Nowadays, barley crop is receiving interest for preparing functional foods as a source of dietary constituents. Besides nutritional attributes, it is a good source of phytochemicals like phenols, flavonols, and tocopherols. Phenolic compounds include benzoic and cinnamic acid derivatives, proanthocyanidins, quinones, flavonols, flavones, flavanones, and amino phenolic compounds (4). The abundance of bioactive compounds confirms the barley's role in preventing many diseases and promoting health (5).

Thermal processing is an important part of food industries; to improve the quality and microbial safety of food products. During the past years, extrusion of food has evolved and now it has been a unique area of research. Extrusion processing is used to prepare expanded snacks, pet foods, porridge, and ready-to-eat cereal foods and modify starch (6). This thermal processing alters functional characteristics of starchy foods by pregelatinizing the starch and then followed by starch retrogradation, leading to retrograded resistant starch (RS). The RS formation is majorly dependent on the severity of extrusion parameters (7). The changes brought in the protein and starch contribute to the structure, texture, and mouthfeel of food products. Moreover, polyphenols undergo various changes during extrusion processing and decrease or increase depending on extrusion conditions (8).

The literature about extrusion processing on RS and glycemic index (GI) of extruded barley flour is still limited. Its nutritional profile makes barley an ideal candidate for developing a new extruded–expanded high fiber ready-to-eat snack food for example with health advantages. The research has been driving to see how extrusion processing factors affected system characteristics as well as physical qualities of barley flour extrudates (expansion, bulk density, texture, and color) until now (3, 9). Therefore, the present study's objective is to formulate barley flour extrudates and the effect of extrusion on RS, GI, and antioxidant properties of barley flour.

MATERIALS AND METHODS

Six cultivars (cv.BH-393, cv.BH-885, cv.BH-902, cv.BH-932, cv.DWR-52, and cv.PL-172) of barley were obtained from CCSHAU, Hisar, India. Cv. DWR-52 and cv.BH-885 were two-rowed and others barley cultivars were of six-rowed.

De-husking, Milling, and Extrusion of Barley

Barley grains were de-husked using a rice polisher. Hulled barley grains were kept in the polisher chamber and run till the hull was removed entirely from the grains. Further, dehulled grains were ground in a Super Mill (Newport Scientific, Australia) and conditioned to 30% moisture content. The flours were packaged in polybags and equilibrated for 12 h. The extrusion of barley flours was carried out on a corotating twin-screw extruder (Clextral, Firminy, France). The screw and the diameter of 25 and 6 mm and (L/D) of 16 were adjusted with the feed rate of 20 kg/h and screw speed of 400 rpm. Barley grains were extruded at 150, and 180°C with barrel temperature of 50, 100, 125 and 150°C (for 150°C) and 50, 100, 140, and 180°C (for 180°C). An induction heating belt heated the terminal section, and the feeding area was cooled with running water.

Hunter Color Characteristics

Hunter color of extruded barley was performed with a Hunter Colorimeter (Restan VA., USA) fitted with a sensor based on color scale [L^* = darkness (0) or lightness (100); a^* = greenness (–) or redness (+); b^* = blueness (–) or yellowness (+)]. $L^*a^*b^*$ values were measured in triplicate, averaged, and recorded. Redness intensity (RI = a^*/b^*) was calculated for each sample, and correlations between color and sensory data were investigated. Calibration with a white standard plate, and its known $L^*a^*b^*$ values, were completed prior to color testing.

Resistant Starch and Glycemic Index

Resistant starch (RS) of barley flours was measured by adopting the method of Goñi et al. (10). The GI of barley flours was calculated following the process of Goñi et al. (10). In the process, *in vitro* study simulated the enzymatic digestion of starch. Hydrolysis index (HI) was measured by dividing the area under the hydrolysis curve of barley flours to the corresponding area of a reference sample i.e., white bread.

The GI was calculated using the following equation:

$$GI = 39.71 + 0.549\ HI.$$

Total Phenolic Content

Total phenolic content (TPC) of barley flour extrudates was evaluated using the Folin-Ciocalteu phenol reagent method (11). The quantity of TPC in barley extrudate extracts was determined using the standard calibration curve of gallic acid solution. Results were represented as μg gallic acid equivalents/g ($\mu g\ g^{-1}$).

Antioxidant Activity

Antioxidant activity (AOA) of barley grains was calculated by following the published method (12) using the methanolic DPPH solution. Methanol was used as a blank, and absorbance at the wavelength of 515 nm was read at 0 and 30 min.

Antioxidant activity was measured as percentage discoloration:

$$\%AOA = (1 - (A\ of\ extracts_{t=30}/A\ of\ extracts_{t=0})) \times 100.$$

TABLE 1 | Hunter color characteristics of extruded (at 150 and 180°C) and non-extruded barley cultivars.

Barley cultivars	L*	a*	b*	ΔE	RI (a*/b*)
BH-393 150°C	$84.3^{b}_{\downarrow 7.5}$	$1.01^{a}_{\uparrow 71.1}$	$10.18^{b}_{\uparrow 10}$	$85.3^{b}_{\downarrow 6.6}$	0.10
180°C	$72.4^{l}_{\downarrow 20.6}$	$1.63^{m}_{\uparrow 176}$	$11.36^{m}_{\uparrow 22.8}$	$73.9^{l}_{\downarrow 19.1}$	0.14
BH-932 150°C	$86.9^{c}_{\downarrow 2.9}$	$1.52^{c}_{\uparrow 10.1}$	$12.4^{e}_{\uparrow 9.2}$	$87.3^{c}_{\downarrow 2.8}$	0.12
180°C	$82.5^{n}_{\downarrow 7.8}$	$1.83^{n}_{\uparrow 32.6}$	$13.6^{o}_{\uparrow 19.8}$	$83.9^{o}_{\downarrow 6.6}$	0.13
BH-902 150°C	$88.0^{e}_{\downarrow 5}$	$1.13^{a}_{\uparrow 79.3}$	$9.3^{a}_{\uparrow 20}$	$89.4^{f}_{\downarrow 3.9}$	0.12
180°C	$85.1^{p}_{\downarrow 8.1}$	$1.34^{l}_{\uparrow 112}$	$10.6^{l}_{\uparrow 36.7}$	$86.3^{q}_{\downarrow 7.3}$	0.13
BH-885 150°C	$87.3^{d}_{\downarrow 3.4}$	$1.79^{e}_{\uparrow 58.4}$	$12.6^{e}_{\uparrow 9.3}$	$88.7^{d}_{\downarrow 2.3}$	0.14
180°C	$80.3^{m}_{\downarrow 11.1}$	$2.03^{o}_{\uparrow 79.6}$	$13.1^{o}_{\uparrow 13.7}$	$82.8^{n}_{\downarrow 8.8}$	0.15
DWR-52 150°C	$83.6^{a}_{\downarrow 7.7}$	$1.43^{cd}_{\uparrow 38.8}$	$11.47^{d}_{\uparrow 6.9}$	$84.6^{a}_{\downarrow 7.4}$	0.12
180°C	$80.1^{m}_{\downarrow 11.5}$	$1.69^{m}_{\uparrow 64}$	$11.91^{n}_{\uparrow 11.1}$	$81.2^{m}_{\downarrow 11.1}$	0.14
PL-172 150°C	$87.3^{d}_{\downarrow 2.1}$	$1.32^{b}_{\uparrow 22.2}$	$10.72^{c}_{\uparrow 9.2}$	$88.3^{e}_{\downarrow 1.9}$	0.12
180°C	$84.5^{o}_{\downarrow 5.2}$	$1.63^{m}_{\uparrow 50.9}$	$11.3^{m}_{\uparrow 15.1}$	$85.3^{p}_{\downarrow 5.3}$	0.14

a–f superscripts are significantly ($p < 0.05$) different within column for extrusion done at 150°C. l–q are significantly ($p < 0.05$) different within column for extrusion done at 180°C. Subscripts denote the percentage increase (↑) and decrease (↓) from control samples for corresponding properties. L^* = darkness (0) or lightness (100); a^* = redness (+); b^* = yellowness (+), redness intensity ($RI = a^*/b^*$), total difference of color value (ΔE).

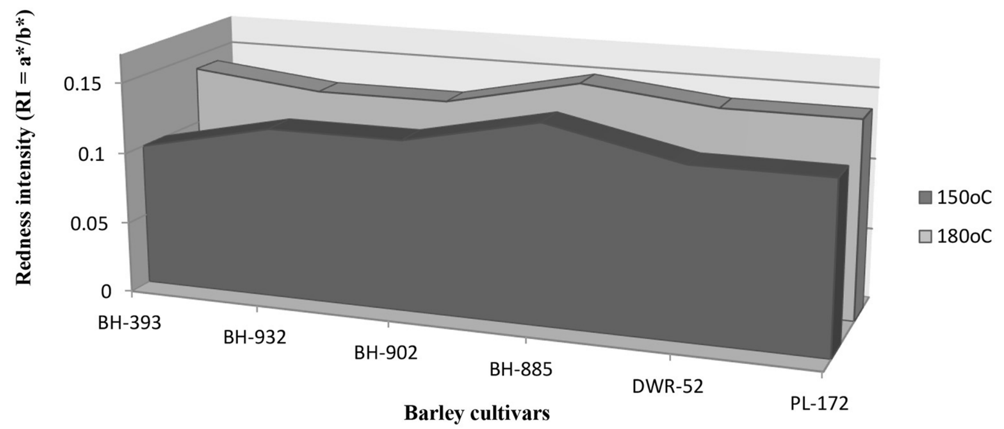

FIGURE 1 | Graphic representation of redness intensity (RI = a*/b*) between barley cultivars.

Total Flavonoids Content

Total flavonoids content (TFC) was measured by adopting the method of Jia et al. (13). The quantity of TFC in barley extrudate extracts was determined using the standard calibration curve of standard catechin solution, and the results were expressed as μg of catechin equivalents (CE)/g.

Metal Chelating Activity

Metal chelating activity (MCA) of extrudates was evaluated by adopting the method of Dinis et al. (14). Metal chelating activity of the samples for $Fe^{2+}$was calculated by using this equation:

Iron (Fe^{2+}) chelating activity (%) = {1 – (Abs of extracts/Abs of control)} × 100.

ABTS (2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic Acid) Radical Cation Decolorization Assay

ABTS+ was calculated by following the already published method (15). The absorbance of extrudates was measured at a wavelength of 734 nm and compared with a standard curve using various concentrations of vitamin C. The result was expressed as vitamin C in μmol/g.

Consumer Studies

Consumers studies of color attributes of barley flour were determined by six panelists (six assessors minimum requested by ISO 11035:2007) from Sensorial Lab, USAMV Cluj-Napoca, both males and females were represented in the panel. All of them have a long experience in product evaluation. Participants used a five-point Likert scale ranging from "dislike extremely" (1) to "like extremely" (7) for the color characteristics obtained by hunter color system.

In a further preliminary evaluation was investigated the role of color in consumers' acceptability and willingness-to-pay (WTP) was investigated. Consumers ($n = 20$) were randomly selected among students and staff of the USAMV Cluj-Napoca university (aged ranged 20–60). The outcome of this study is expected to provide support and guidance to current and future research on barley four extrudates.

TABLE 2 | Resistant starch of extruded (at 150 and 180°C) and non-extruded barley flours.

Barley cultivars	Resistant starch (RS) (%)		
	Non-extruded	150°C	180°C
BH-393	1.52^{b}	$1.87^{b}_{\uparrow 23}$	$2.26^{b}_{\uparrow 48.6}$
BH-932	1.56^{b}	$2.08^{c}_{\uparrow 33.3}$	$2.56^{c}_{\uparrow 64.1}$
BH-902	1.99^{cd}	$2.78^{e}_{\uparrow 39.6}$	$2.96^{e}_{\uparrow 48.7}$
BH-885	1.33^{a}	$1.7^{a}_{\uparrow 27.8}$	$2.16^{a}_{\uparrow 62.4}$
DWR-52	2.2^{d}	$2.34^{d}_{\uparrow 6.3}$	$2.82^{d}_{\uparrow 28.1}$
PL-172	1.93^{c}	$2.15^{cd}_{\uparrow 11.3}$	$2.57^{c}_{\uparrow 33.1}$

a–e superscripts are significantly ($p < 0.05$) different within column for extrusion done at 150°C and 180°C. Subscripts denote the percentage increase (↑) and decrease (↓) from control samples for corresponding properties.

TABLE 3 | Glycemic index of flours from extruded (at 150 and 180°C) and non-extruded barley flours.

Barley cultivars	Resistant starch (RS) (%)		
	Non-extruded	150°C	180°C
BH-393	24.6^{e}	$23.2^{d}_{\downarrow 5.6}$	$22.4^{d}_{\downarrow 8.9}$
BH-932	23.8^{cd}	$22.8^{cd}_{\downarrow 4.3}$	$22.7^{d}_{\downarrow 4.6}$
BH-902	22.6^{b}	$21.9^{bc}_{\downarrow 3.0}$	$20.3^{b}_{\downarrow 10.1}$
BH-885	24.1^{d}	$22.6^{c}_{\downarrow 6.2}$	$22.1^{c}_{\downarrow 8.2}$
DWR-52	21.2^{a}	$20.6^{a}_{\downarrow 2.8}$	$19.2^{a}_{\downarrow 9.4}$
PL-172	23.3^{c}	$21.2^{b}_{\downarrow 9.0}$	$19.3^{a}_{\downarrow 17.1}$

a–e superscripts are significantly ($p < 0.05$) different within column for extrusion done at 150°C and 180°C. Subscripts denote the percentage increase (↑) and decrease (↓) from control samples for corresponding properties.

TABLE 4 | Total phenolic content (TPC, μg GAE/g) of extruded (at 150 and 180°C) and non-extruded barley flours.

Barley cultivars	Non-extruded	150°C	180°C
BH-393	3256^{d}	$2246^{c}_{\downarrow 31}$	$2013^{c}_{\downarrow 38.1}$
BH-932	3056^{c}	$2348^{d}_{\downarrow 23.1}$	$2045^{c}_{\downarrow 33}$
BH-902	3761^{e}	$3108^{e}_{\downarrow 17.3}$	$2879^{e}_{\downarrow 23.4}$
BH-885	3922^{f}	$3277^{f}_{\downarrow 16.4}$	$2756^{d}_{\downarrow 29.7}$
DWR-52	2922^{b}	$1935^{b}_{\downarrow 33.7}$	$1845^{b}_{\downarrow 36.8}$
PL-172	2890^{a}	$1899^{a}_{\downarrow 34.2}$	$1788^{a}_{\downarrow 38.1}$

a–e superscripts are significantly ($p < 0.05$) different within column for extrusion done at 150°C and 180°C. Subscripts denote the percentage increase (↑) and decrease (↓) from control samples for corresponding properties.

Consumers were asked to complete a choice-based conjoint analysis to determine their WTP for barley flours obtained with different color characteristics indicated by hunter color system and health benefits indicated by results of analysis performed in this study."

Price for barley flours was varied at three levels. Every choice question contained two options: (A) "I am willing to pay the price of a commercial barley flour" and (B) "I am willing to pay a higher price than a commercial barley flour, "plus a (C) "no buy" option.

Data Analysis

The results generated during the analysis of barley flour extrudates was an average of three independent observations, which were further screened through ANOVA using the Minitab software ver. 16, and one-way ANOVA with *post-hoc* Tukey HSD Test. Results are presented in **Table 7.**

RESULTS AND DISCUSSION

Color Parameters

The extruded barley cultivars were evaluated for their color characteristics by hunter color system (**Table 1**). The L^* and ΔE values of extruded samples reduced significantly ($p <$ 0.05) when compared with control counterparts (data already published) and the values ranged from 83.6 to 88 and 84.6 to 89.4, respectively, for extrusion done at 150°C, and from 72.4 to 85.1 and 73.9 to 86.3, respectively, for extrusion done at 180°C. A reduction in L^* and ΔE-value could be due to the initiation and propagation of the Maillard reaction during extrusion resulting in producing brown pigments (16). It is evidenced that the L^* value reduced with increasing barrel temperature (B) while enhanced with increasing moisture content. High temperature and low moisture content favored the Maillard reaction (17). Maillard pigments contribute to the color, aroma, and flavor of food goods. The a^*-value differed significantly ($p < 0.05$) among barley cultivars and increased after extrusion. It ranged from 1.01 to 1.79 and 1.34 to 2.03 for extrusion done at 150 and 180°C, respectively. The b^*-value of flours from extruded barley cultivars also increased and differed significantly within the cultivars, with values of 9.3–12.6 (at 150°C) and 10.6–13.6 (at 180°C). An increase in redness and yellowness could also be attributed to Maillard products influenced by temperature, moisture content, reactant concentration, and reaction time (18).

Overall, RI increased from 150°C (range: 0.41–0.57) to 180°C (range: 0.51–0.60) (**Table 1**; **Figure 1**), and differences between cultivars are representative.

Resistant Starch

Resistant starch in barley flour extrudates was calculated in the range of 1.7–2.78% at 150°C and 2.16–2.96% at 180°C compared to 1.33–2.2% recorded in barley flour non-extrudates (**Table 2**). Gulzar et al. (17) also recorded an enhancement of 4.91–6.83% in RS content of extruded rice flour. During extrusion, heat, and moisture content alter starch characteristics simultaneously, leading to interacting with non-starch components, thus supporting the retrograded starch production (17). Moisture behaves as a plasticizer for the starch retrogradation process, and at 30–60% moisture content, retrogradation is at its maximum (19). It is depicted from the results that there was a corresponding increase in RS content with increasing temperature and moisture. It is reported that the production of starch lipid complexes during the extrusion process, resulting in the formation of RS, which has the potential to lower starch digestibility (20).

TABLE 5 | Antioxidant potentials of extruded (at 150 and 180°C) and non-extruded barley flours.

Barley cultivars	DPPH (%)			MCA (%)			ABTS^{+} (μmol/g)		
	Non-extruded	150°C	180°C	Non-extruded	150°C	180°C	Non-extruded	150°C	180°C
BH-393	21.3^{c}	$23.3^{b}_{\uparrow 9.3}$	$27.3^{c}_{\uparrow 28.1}$	44^{c}	$58.4^{d}_{\uparrow 32.7}$	$64.2^{d}_{\uparrow 45.9}$	15.4^{b}	$17.4^{b}_{12.9\uparrow}$	$19.9^{bc}_{29.2\uparrow}$
BH-932	20.5^{bc}	$24.7^{c}_{\uparrow 20.4}$	$26.8^{b}_{\uparrow 30.7}$	38^{b}	$56.6^{c}_{\uparrow 48.9}$	$59.2^{b}_{\uparrow 55.7}$	17.8^{d}	$20.2^{d}_{13.4\uparrow}$	$21.3^{cd}_{19.6\uparrow}$
BH-902	24.9^{d}	$29.9^{d}_{\uparrow 20}$	$33.6^{e}_{\uparrow 34.9}$	38^{b}	$55.2^{b}_{\uparrow 45.2}$	$60.2^{c}_{\uparrow 58.4}$	16.8^{c}	$19.2^{c}_{14.2\uparrow}$	$20.8^{c}_{23.8\uparrow}$
BH-885	25.8^{e}	$29.2^{d}_{\uparrow 13.1}$	$32.7^{d}_{\uparrow 26.7}$	51^{d}	$61.4^{f}_{\uparrow 20.3}$	$69.0^{f}_{\uparrow 35.2}$	15.1^{b}	$20.6^{d}_{36.4\uparrow}$	$22.2^{d}_{47\uparrow}$
DWR-52	19.8^{b}	$24.8^{c}_{\uparrow 25.2}$	$27.1^{bc}_{\uparrow 36.8}$	42^{c}	$59.9^{e}_{\uparrow 42.6}$	$65.2^{e}_{\uparrow 55.2}$	13.2^{a}	$15.6^{a}_{18.1\uparrow}$	$17.1^{a}_{29.5\uparrow}$
PL-172	18.3^{a}	$21.9^{a}_{\uparrow 19.6}$	$25.7^{a}_{\uparrow 40.4}$	31^{a}	$47.6^{a}_{\uparrow 53.5}$	$53.4^{a}_{\uparrow 72.2}$	15.3^{b}	$17.5^{b}_{14.3\uparrow}$	$19.1^{b}_{24.8\uparrow}$

a–e superscripts are significantly ($p < 0.05$) different within column for extrusion done at 150°C and 180°C. Subscripts denote the percentage increase (↑) and decrease (↓) from control samples for corresponding properties.

TABLE 6 | Total flavonoids content TFC (μg CE/g) of extruded (at 150 and 180°C) and non-extruded barley flours.

Barley cultivars	Non-extruded	150°C	180°C
BH-393	2011^{b}	$1{,}602^{d}_{\downarrow 20.3}$	$1{,}177^{d}_{\downarrow 41.4}$
BH-932	2024^{b}	$1{,}569^{c}_{\downarrow 22.4}$	$1{,}033^{c}_{\downarrow 48.9}$
BH-902	1988^{ab}	$1{,}222^{a}_{\downarrow 38.5}$	$946^{b}_{\downarrow 52.4}$
BH-885	2198^{c}	$1{,}835^{e}_{\downarrow 16.5}$	$1{,}339^{e}_{\downarrow 39}$
DWR-52	2002^{b}	$1{,}546^{c}_{\downarrow 22.7}$	$1{,}033^{c}_{\downarrow 48.4}$
PL-172	1968^{a}	$1{,}299^{b}_{\downarrow 33.9}$	$898^{a}_{\downarrow 54.3}$

a–e superscripts are significantly ($p < 0.05$) different within column for extrusion done at 150°C and 180°C. Subscripts denote the percentage increase (↑) and decrease (↓) from control samples for corresponding properties.

Formation of starch-lipid complexes can be identified by the V-type x-ray diffraction pattern. As a low-calorie component, RS became the interest of food enterprises and dietitians. It improves the food structure and shifts the consumption trend to healthy and nutritious food with nutrition and health care functions. Resistant starch could help in the prevention of increased blood sugar and avoid cancers and cardiovascular diseases (21).

Glycemic Index

The GI of barley flour extrudates was found to be in the range of 20.6–23.3 at 150°C and 19.2–22.7% at 180°C and was significantly lower than recorded in barley flour non-extrudates (21.1–24.6%) (**Table 3**). Barley flour extrudates recorded a significant increase in RS with increased temperature, which likely reduced the GI-value. It is reported that the extent of decrease in GI-value is more pronounced at high moisture content. Extrusion of starchy foods at high moisture content leads to starch gelatinization and alteration of the native structure (17). Extrusion followed by drying results in the reorganization of the crystalline domain within the amorphous domain. This reorganization resulted in retrogradation results in a decrease in GI and an increase in RS content. These findings agree with previous studies of Hussain et al. (22), where an enhancement in RS with the reduced GI in preconditioned water chestnut flour was observed.

In the actual context of growing trends in high RS and low GI diets, can be concluded from this study that extrusion of barley at 150 and 180°C is a processing method of great importance in enhancing of RS and in the same time reducing the GI.

Total Phenolic Content

Total phenolic content (TPC) of non-extruded barley flours ranged between 2,890 and 3,922 μg GAE/g. A decrease in TPC values was observed upon extrusion at 180 and 150°C temperature. Total phenolic content in barley extrudates at 180°C ranged from 1,788 to 2,879 μg GAE/g with the greatest and lowest for cv. BH-902 and cv. PL-172. In extrudates at 150°C, TPC differed significantly among barley cultivars and varied between 1,899 to 3,277 μg GAE/g (**Table 4**). Similar results were also observed on the TPC of sorghum extrudates (23). The authors observed a decrease of 11.8–20.0% in the free phenolic content of sorghum. However, Ramos-Enríquez et al. (24) evaluated the impact of extrusion processing on wheat bran at 140 and 180°C at 30% moisture content and observed an enhancement of bound TPC. In conclusion, the extrusion process either cause degradation and destruction of the molecular structure of heat-labile phenolic compounds or disintegrate the cell wall matrix and breaks covalent bonding of phenolics resulting in improved phenolic compounds (25–27). Besides, moisture content also affects phenolic content; at low moisture content, the shear force is higher, resulting in phenolics more prone to thermal degradation (28).

Antioxidant Potentials

Determination of 1,1-diphenyl-2-picrylhydrazyl Radical Scavenging Activity

For the evaluation of the antioxidant potentialradical 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity has been used to compare antioxidants, which quantifies the hydrogen donation potential of the compounds. The DPPH radical scavenging activity in the non-extruded barley flours ranged from 18.3 to 25.8% with cv.BH-885 and cv.PL-172 having the greatest and the lowest DPPH radical scavenging activity. The extrusion process led to a significant improvement in DPPH in all barley flour extrudates compared to non-extruded counterparts. An increase in DPPH could be due to the breaking of the covalent bond of the cell wall resulting in increased phenolic content

TABLE 7 | One-way ANOVA with *post-hoc* Tukey HSD Test.

	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
Resistant starch			
Non-extruded vs. 150°C	2.8533	0.1419605	NS
Non-extruded vs. 180°C	5.7305	0.0028074	$^{*}p < 0.01$
150 vs. 180°C	2.8772	0.1379327	NS
Glycemic index^{+}			
Non-extruded vs. 150°C	2.2998	0.2655477	NS
Non-extruded vs. 180°C	4.2846	0.0216293	$^{**}p < 0.05$
150 vs. 180°C	1.9848	0.3647908	NS
TPC			
Non-extruded vs. 150	4.0349	0.030	$^{+}p < 0.05$
Non-extruded vs. 180	5.2363	0.005	$^{++}p < 0.01$
150 vs. 180°C	1.2014	0.669	NS
DPHH			
Non-extruded vs. 150	2.9691	0.123	NS
Non-extruded vs. 180	5.4519	0.004	$\pm p < 0.01$
150 vs. 180°C	2.4828	0.217	NS
MCA			
Non-extruded vs. 150	6.7487	0.001	$^{\emptyset}p < 0.01$
Non-extruded vs. 180	9.0266	0.001	$^{\emptyset}p < 0.01$
150 vs. 180°C	2.2779	0.271	NS
ABTS^{+}			
Non-extruded vs. 150	8.8792	0.001	$^{§}p < 0.01$
Non-extruded vs. 180	7.7301	0.001	$^{§}p < 0.01$
150 vs. 180°C	1.1491	0.690	NS
TFC			
Non-extruded vs. 150	7.6732	0.001	#$p < 0.01$
Non-extruded vs. 180	14.1873	0.001	#$p < 0.01$
150 vs. 180°C	6.5141	0.001	#$p < 0.01$

NS, not statistically significant.

[*] *The p = 0.003 < 0.05 corresponding to the F-stat = 8.20 of one-way ANOVA, suggesting that the samples are significantly different.*

[**] *The p = 0.02 < 0.05 corresponding to the F-stat = 4.59 of one-way ANOVA, suggesting that the samples are significantly different.*

[+] *The p = 0.005 < 0.05 corresponding to the F-stat = 7.52 of one-way ANOVA, suggesting that the one or more sample are significantly different.*

[±] *The p = 0.005 < 0.05 corresponding to the F-stat = 7.45 of one-way ANOVA, suggesting that the one or more sample are significantly different.*

[∅] *The p = 0.000 < 0.05 corresponding to the F-stat = 22.03 of one-way ANOVA, suggesting that the one or more sample are significantly different.*

[§] *The p = 0.000 < 0.05 corresponding to the F-stat = 23.31 of one-way ANOVA, suggesting that the one or more sample are significantly different.*

[#] *The p = 0.000 < 0.05 corresponding to the F-stat = 50.43 of one-way ANOVA, suggesting that the one or more sample are significantly different.*

and enhanced AOA (29), or the production of brown pigments (particularly melanoidins) due to the Maillard reaction (30). The production of browning pigments resulted in improved DPPH radical scavenging activity (16). 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity differed significantly among barley flour extruded at 150°C with 30% moisture. The DPPH radical scavenging activity ranged from 21.9% to 29.9%, with the greatest and lowest for cv. BH-902 and cv. PL-172, respectively. Similarly, at 180°C, the DPPH radical scavenging activity varied between 25.7 and 33.6% (**Table 5**). The BH-902 exhibited the greatest, and PL-172 exhibited the lowest DPPH radical scavenging activity. Delgado-Nieblas et al. (31) also reported the enhanced DPPH radical scavenging activity in breakfast cereals as the extrusion temperature was increased.

Metal Chelating Activity

Metal Chelating Activity (MCA %) is the most common method used for evaluating the antioxidant potential of substances. In the barley flours, MCA varied from 31 to 51% (**Table 5**). Upon extrusion, MCA increased in all the barley cultivars as compared to their non-extruded parts. Extrusion at 150°C with feed moisture of 30% improved the MCA, and the values ranged from 47.6 to 61.4%. cv. BH-885 showed the greatest and cv. PL-172 showed the lowest MCA. Sharma et al. (8) observed an increase in MCA of extruded barley up to 23%. They observed that the rise in MCA is due to novel substances (melanoidins) during thermal processing. However, with an increase in 150 to 180°C temperature with constant moisture content of 30%, improved MCA was observed. The greatest and the lowest MCA exhibited cv.BH-885 (69%) and cv.PL-172 (53.4%) (**Table 5**). Improved MCA upon increasing temperature and feed moisture could be due to the production of Maillard compounds with different concentrations. Moreover, produced Maillard compounds are melanoidins (high MW) and heterocyclic compounds (low MW), which are responsible for DPPH radical scavenging activity and MCA.

ABTS (2, 2′-azinobis 3-ethylbenzothiazoline-6-sulfonic Acid) Radical Cation Decolorization Assay

To measure the potential of antioxidants to quench the ABTS^{+} radicals, the reaction between antioxidants with ABTS^{+} occurs rapidly and can be assessed by following the reduced absorbance of samples at 734 nm. The AOA in the barley cultivars ranged from 13.2 to 17.8 μmol/g (**Table 5**). The extrusion cooking showed an increase in ABTS^{+} scavenging activity; however, a non-significant difference in barley extrudates at 150 and 180°C was observed. The ABTS^{+} activity of barley extrudates ranged from 15.6 to 20.6 μmol/g at 150°C and between 17.1 and 21.3 μmol/g at 180°C (**Table 5**). Results support the fact that thermal processing alters the antioxidant profile and produces more antioxidants contributing to AOA. Delgado-Nieblas et al. (31) reported an increase in ABTS^{+} scavenging activity of extruded cereal breakfast prepared from wheat and oat bran, yellow corn grits incorporated with naranjita pomace. It has been demonstrated that cereals have the potential to scavenge ABTS^{+} radicals, with potential effects in the reduction of lipid peroxidation (32).

Total Flavonoids Content

Flavonoids have been associated to a variety of health benefits, including antioxidant potential benefits. Total flavonoids content (TFC) in the six barley cultivars varied from 1,968 to 2,198 μg CE/g. Upon extrusion, a significant ($p < 0.05$) reduction in the TFC was reported. The TFC of barley extrudates (150°C and 30% moisture content) different significantly among barley cultivars

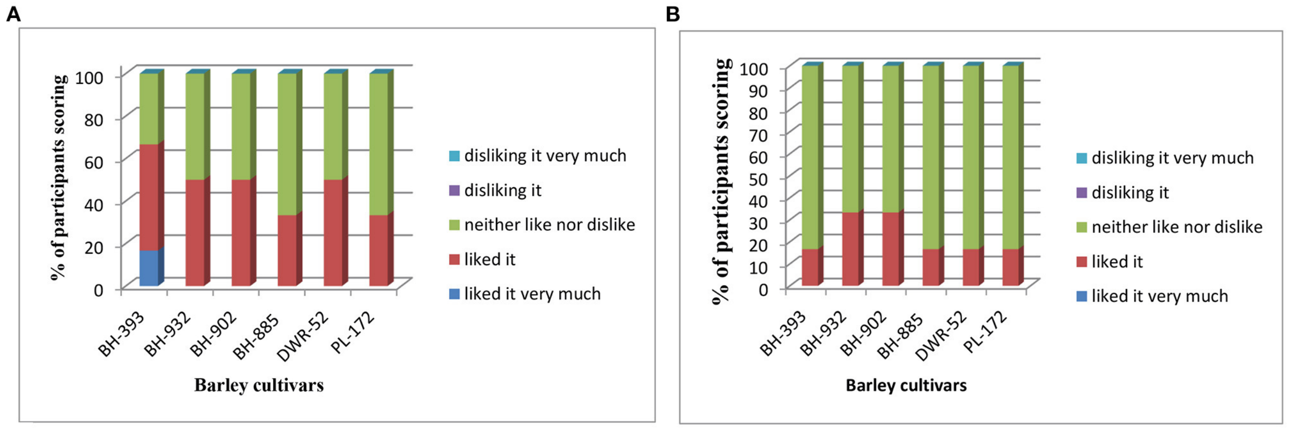

FIGURE 2 | Results of the panelists tests (frequencies of scores for overall evaluation) for redness intensity (RI = a^*/b^*) between barley cultivars **(A)** extruded at 150°C, and **(B)** extruded at 180°C.

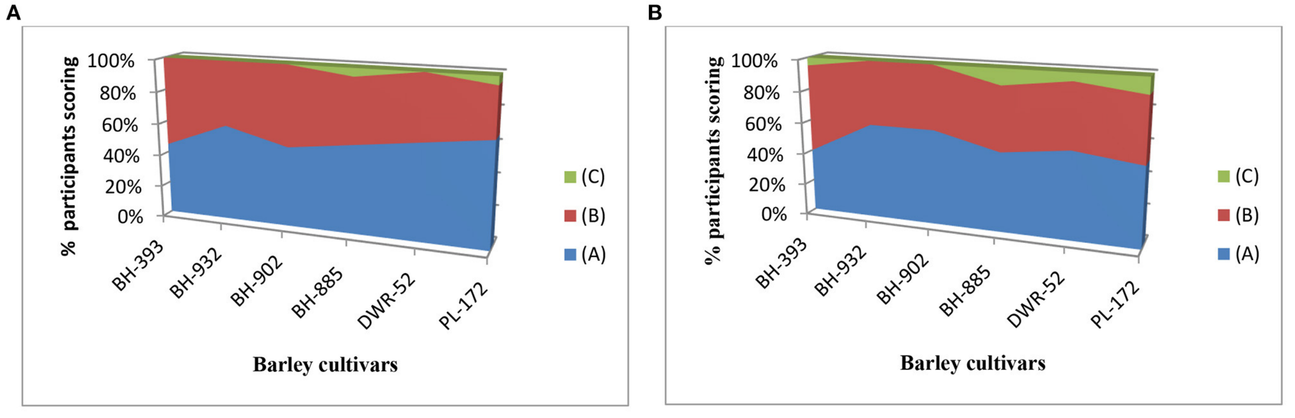

FIGURE 3 | Results of the consumers studies willingness-to-pay (WTP) (frequencies of scores for overall evaluation) of barley cultivars **(A)** at 150°C, and **(B)**. **(A)** "I am willing to pay the price of a commercial barley flour, **(B)** "I am willing to pay a higher price than a commercial barley flour," **(C)** "no buy."

and ranged from 1,222 to 1,835 μg CE/g; BH-885 exhibited the greatest, and BH-902 showed the lowest TFC (**Table 6**). With increasing temperature from 150 to 180°C with the moisture content of 30%, a significant ($p < 0.05$) decrease was observed in TFC. Total flavonoids content ranged from 898 to 1,177 μg CE/g/g with the greatest and the lowest for BH-393 and PL-172. Zhang et al. (33) evaluated TFC of 0.992 mg rutin/g of buckwheat flour lowered by 33% after thermal treatment. A reduction in TFC could be due to the degradation of heat-sensitive flavonoids (34, 35).

Antioxidant supplements, according to evidence, do not work as effectively as antioxidants found naturally in foods. Antioxidant-rich foods may lower the risk of a variety of diseases (including certain cancers) (36). The present research is proving that barley extrudates are rich sources of antioxidants offering health benefits if integrated in a regularly diet from early life to be effective.

Data Analysis

The results are presented in **Table 7** below.

Consumers Tests

The panelists tests have showed some differences between the evaluation of at 150 and 180°C (**Figure 2**). Overall, all barley cultivars flours extruded scored between liked it or liked it very much almost same as neither like nor dislike. This result could be valuable for further applications. At 150°C flour extruded scored average 40.47% of panelists, liked it or liked it very much, compared to 52.78% of panelists neither like nor dislike. At 180°C flour extruded scored average 22.27% of panelists, liked it only, compared to 77.73% of panelists neither like nor dislike.

Furthermore, the choice-based conjoint analysis conducted for the barley cultivars at 150°C showed (**Figure 3**) that 55.83% consumers are willing to pay the price of a commercial barley flour and 42.5% are willing to pay a higher price than a commercial barley flour, relative to 1.66% not buying. Compared to choice-based conjoint analysis conducted for the barley cultivars at 180°C showed that 52.50% consumers are willing to pay the price of a commercial barley flour and same 42.5% are willing to pay a higher price than a commercial barley flour, relative to 5.0% not buying.

It is still relevant to mention that color and appearance of the food product is an important aspect considered by the consumers. If the food product deviates from the expected color, it can have an impact on consumer choices buy it or not. The discoloration of bread by barley in general having a dark gray color is one of the obstacles that prevent the use of barley in food products, and even when it is used in combination with other flour types, such as rice or wheat. It has been proven that the polyphenol content and level of polyphenol oxidase activity varies dramatically between different barley genotypes. The phenolic compounds are mainly found in the hull, testa, and aleurone of cereal grains, compared to barley.

Several solutions to reduce the discoloration of barley-containing foods have been proposed, such as heat-treatment in order to denature the polyphenol oxidase of barley flour resulting in less discoloration compared to unheated barley flour. However, these kind of processes result in a reduction of all polyphenolic content in the barley flour and decrease their health-promoting value.

Another alternative proposed is further research to identify which specific polyphenols have antioxidant properties (37), so that the development of barley genotypes that lack the discoloring polyphenols but have a high antioxidant polyphenol count can occur. Antioxidants have recently gained significant consideration, and one of the cereals containing most antioxidants is barley. People going to gym and train out, on the other hand, require bakery products to maintain a balanced diet. Looking at this trend the bakery and confectionary segments shall dominate the end-user category of global barley flour market between 2019 and 2029 (38).

CONCLUSIONS

Extrusion has evolved as highly promising thermal processing method for producing foods and food ingredients. Extrusion processing positively affected the RS content significantly higher and GI significantly lower than recorded in barley flour non-extrudates of six barley cultivars tested. Barley subjected to high temperature formed brown pigments, which promote the antioxidant activities of barley flours. The results of present study demonstrated that the extrusion could be adopted to improve the barley flour quality, and therefore barley-rich functional foods with increased antioxidant activities can be develop and formulate, especially that the consumers' studies have shown the acceptance of the improved barley flour. The antioxidant benefits is well-recognized, and appropriate antioxidant nutrition plays an significant role in the development of functional food products tailored to certain life phases, lifestyles, or activity levels, as well as the support of food products functionality addressing specific health or wellbeing concerns.

CODE AVAILABILITY STATEMENT

MS office-2016 and ANOVA using the Minitab software ver. 16.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SP and AR drafted the manuscript. MK proofread the manuscript. All authors participated in the performing, generating and interpretation of results.

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REFERENCES

1. FAO—Food and Agriculture Organization of the United Nations. *FAOSTAT Statistics Database-Agriculture*. Rome: FAO (2017).
2. Dietrich T, Velasco MV, Echeverría P, Pop B, Rusu A. Crop and plant biomass as valuable material for BBB. Alternatives for valorization of green wastes. In: *Biotransformation of Agricultural Waste and By-Products: The Food, Feed, Fiber, Fuel (4F) Economy*. San Diego, CA: Elsevier. (2016). doi: 10.1016/B978-0-12-803622-8.00001-X
3. Lotfi Shirazi S, Koocheki A, Milani E, Mohebbi M. Production of high fiber ready-to-eat expanded snack from barley flour and carrot pomace using extrusion cooking technology. *J Food Sci Technol.* (2020) 57:2169–81. doi: 10.1007/s13197-020-04252-5
4. Holtekjølen AK, Bævre AB, Rødbotten M, Berg H, Knutsen SH. Antioxidant properties and sensory profiles of breads containing barley flour. *Food Chem*. (2008) 110:414–21. doi: 10.1016/j.foodchem.2008.02.054
5. Baba WN, Rashid I, Shah A, Ahmad M, Gani A, Masoodi FA, et al. Effect of microwave roasting on antioxidant and anticancerous activities of barley flour. *J Saudi Soc Agric Sci*. (2016) 15:12–9. doi: 10.1016/j.jssas.2014.06.003
6. Alam MS, Kaur J, Khaira H, Gupta K. Extrusion and extruded products: changes in quality attributes as affected by extrusion process parameters: a review. *Crit Rev Food Sci Nutr*. (2016) 56:445–73. doi: 10.1080/10408398.2013.779568
7. Ai Y, Cichy KA, Harte JB, Kelly JD, Ng PK. Effects of extrusion processing on the chemical composition and functional properties of dry common bean powders. *Food Chem.* (2016) 211:538–45. doi: 10.1016/j.foodchem.2016.05.095
8. Sharma P, Gujral HS, Singh B. Antioxidant activity of barley as affected by extrusion cooking. *Food Chem.* (2012) 131:1406–13. doi: 10.1016/j.foodchem.2011.10.009
9. Liu Y, Liu M, Huang S, Zhang Z. Optimisation of the extrusion process through a response surface methodology for improvement of the physical

properties and nutritional components of whole black-grained wheat flour. *Foods (Basel, Switzerland).* (2021) 10:437. doi: 10.3390/foods10020437
10. Goñi I, Garcia-Alonso A, Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. *Nutr Res.* (1997) 17:427–37. doi: 10.1016/S0271-5317(97)00010-9
11. Gao L, Wang S, Oomah BD, Mazza G. Wheat quality: antioxidant activity of wheat millstreams. In: Wheat Quality Elucidation, P. Ng, and C. Wrigley W, editor. St. Paul, MN: American Association of Cereal Chemists (2002). p. 219–33.
12. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol.* (1995) 28:25–30. doi: 10.1016/S0023-6438(95)80008-5
13. Jia Z, Tang M, Wu J. The determination of flavonoids content in mulberry and their scavenging effects on superoxides radicals. *Food Chem.* (1998) 64:555–9. doi: 10.1016/S0308-8146(98)00102-2
14. Dinis TCP, Madeira VMC, Almeidam LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxylradicals scavengers. *Arch Biochem Biophys*. (1994) 315:161–9. doi: 10.1006/abbi.1994.1485
15. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* (1999) 26:1231–7. doi: 10.1016/S0891-5849(98)00315-3
16. Rufián-Henares JA, Delgado-Andrade C. Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. *Food Res Int.* (2009) 42:394–400. doi: 10.1016/j.foodres.2009.01.011
17. Gulzar B, Hussain SZ, Naseer B, Naik HR. Enhancement of resistant starch content in modified rice flour using extrusion technology. *Cereal Chem.* (2021) 98:634–41. doi: 10.1002/cche.10407
18. Stojceska V, Ainsworth P, Plunkett A, Ibanoglu S. The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. *Food Chem.* (2009) 114:226–32. doi: 10.1016/j.foodchem.2008.09.043
19. Jang JK, Pyun YR. Effect of sucrose and gluten on glass transition, gelatinization, and retrogradation of wheat starch. *Kor J Food Sci Technol.* (2004) 36:288–93.
20. Raigond P, Ezekiel R, Raigond B. Resistant starches in food: a review. *J Sci Food Agric.* (2015) 95:1968–78. doi: 10.1002/jsfa.6966
21. Tian S, Sun Y. Influencing factor of resistant starch formation and application in cereal products: a review. *Int J Biol Macromol.* (2020) 149:424–31. doi: 10.1016/j.ijbiomac.2020.01.264
22. Hussain SZ, Beigh MA, Naseer B, Amin T, Naik HR. Characteristics of resistant starch in water chestnut flour as improved by preconditioning process. *Int J Food Properties.* (2019) 22:449–61. doi: 10.1080/10942912.2019.1588300
23. de Morais Cardoso L, Pinheiro SS, de Carvalho CWP, Queiroz VAV, de Menezes CB, Moreira AVB, et al. Phenolic compounds profile in sorghum processed by extrusion cooking and dry heat in a conventional oven. *J Cereal Sci.* (2015) 65:220–6. doi: 10.1016/j.jcs.2015.06.015
24. Ramos-Enríquez JR, Ramírez-Wong B, Robles-Sánchez RM, Robles-Zepeda RE, González-Aguilar GA, Gutiérrez-Dorado R. Effect of extrusion conditions and the optimization of phenolic compound content and antioxidant activity of wheat bran using response surface methodology. *Plant Foods for Human Nutrition.* (2018) 73:228–34. doi: 10.1007/s11130-018-0679-9
25. Brennan C, Brennan M, Derbyshire E, Tiwari BK. Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends Food Sci Technol.* (2011) 22:570–5. doi: 10.1016/j.tifs.2011.05.007
26. Ti H, Zhang R, Zhang M, Wei Z, Chi J, Deng Y, et al. Effect of extrusion on phytochemical profiles in milled fractions of black rice. *Food Chem.* (2015) 178:186–94. doi: 10.1016/j.foodchem.2015.01.087
27. Mitrea L, Călinoiu LF, Precup G, Bindea M, Rusu B, Trif M, et al. Inhibitory potential of *Lactobacillus plantarum* on *Escherichia coli*. *Bull UASVM Food Sci Technol.* (2017) 74:99–101. doi: 10.15835/buasvmcn-fst:0031
28. Ortiz-Cruz RA, Ramírez-Wong B, Ledesma-Osuna AI, Torres-Chávez PI, Sánchez-Machado DI, Montaño-Leyva B, et al. Effect of extrusion processing conditions on the phenolic compound content and antioxidant capacity of sorghum (*Sorghum bicolor* (L.) Moench) Bran. *Plant Foods Hum Nutr.* (2020) 75:252–7. doi: 10.1007/s11130-020-00810-6
29. Taylor JR, Duodu KG. Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. *J Sci Food Agric.* (2015) 95:225–37. doi: 10.1002/jsfa.6713
30. Randhir R, Kwon Y-I, Lin YT, Shetty K. Effect of thermal processing on the phenolic associated health-relevant functionality of selected legume sprouts and seedlings. *J Food Biochem.* (2009) 33:89–112. doi: 10.1111/j.1745-4514.2008.00210.x
31. Delgado-Nieblas C, Ruiz-Beltrán K, Sánchez-Lizárraga J, Zazueta-Morales JDJ, Aguilar-Palazuelos E, Carrillo-López A, et al. Effect of extrusion on physicochemical, nutritional and antioxidant properties of breakfast cereals produced from bran and dehydrated naranjita pomace. *CyTA J Food.* (2019) 17:240–50. doi: 10.1080/19476337.2019.1566276
32. Zielinski H, Kozłowska H. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J Agric Food Chem.* (2000) 48:2008–16. doi: 10.1021/jf990619o
33. Zhang M, Chen H, Li J, Pei Y, Liang Y. Antioxidant properties of tartarybuckwheat extracts as affected by different thermal processing. *LWT-Food Sci Technol.* (2010) 43:181–5. doi: 10.1016/j.lwt.2009.06.020
34. Xu B, Chang SK. Effect of soaking, boiling, and steaming on total phenolic contentand antioxidant activities of cool season food legumes. *Food Chem.* (2008) 110:1–13. doi: 10.1016/j.foodchem.2008.01.045
35. Szabo K, Dulf FV, Teleky B-E, Eleni P, Boukouvalas C, Krokida M, et al. Evaluation of the bioactive compounds found in tomato seed oil and tomato peels influenced by industrial heat treatments. *Foods.* (2021) 10:110. doi: 10.3390/foods10010110
36. Jurj A, Pop LA, Zanoaga O, Ciocan-Cârtită CA, Cojocneanu R, Moldovan C, et al. New Insights in gene expression alteration as effect of paclitaxel drug resistance in triple negative breast cancer cells. *Cell Physiol Biochem.* (2020). 54:648–64. doi: 10.33594/000000246
37. Rusu AV, Criste FL, Mierlită D, Socol CT, Trif M. Formulation of lipoprotein microencapsulated beadlets by ionic complexes in algae-based carbohydrates. *Coatings.* (2020) 10:302. doi: 10.3390/coatings 10030302
38. Bindereif SG, Rüll F, Kolb P, Köberle L, Willms H, Steidele S, et al. Impact of global climate change on the european barley market requires novel multi-method approaches to preserve crop quality and authenticity. *Foods (Basel, Switzerland).* (2021) 10:1592. doi: 10.3390/foods 10071592

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Food Consumption Frequency, Perceived Stress, and Depressive Symptoms Among Female University Students in Dubai, United Arab Emirates

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Background: This study assessed whether perceived stress and depressive symptoms were associated with the frequency of consumption of specific food groups among female university students.

Methods: A cross-sectional study was conducted among female university students using a simple random sampling method. The response rate was 97%, with a total number of 385 participants. The associations between stress levels and most/least-consumed food groups, and between depressive symptoms levels and most/least-consumed food groups were assessed. The questionnaire included a 12-item self-administered food frequency questionnaire, Cohen's Perceived Stress Scale, and the Beck Depression Inventory-II. The study was approved by the University Ethical Committee prior to the data collection. One-way Analysis of Variance (ANOVA) and an independent-sample *t*-test were performed to test the equality of population means across the categories of each independent variable depending on the number of categories of the independent variable.

Results: Overall, this group of female university students fell under the mild mood disturbance category (depressive symptoms) (BDI-II) and had moderate perceived stress (PSS). Perceived stress was associated with more frequent consumption of salad/raw vegetables and cooked vegetables and less frequent consumption of cake/cookies and meat/sausage products ($p < 0.05$). Additionally, depressive symptoms were associated with less frequent consumption of fresh fruits and increased consumption of fast food/canned food and soft drinks ($p < 0.05$).

Conclusions: The data showed that stress and depression were associated with different dietary preferences, which is consistent with the distinctions between stress and depression in human behavior. Specifically, the results revealed associations between soft drinks consumption and higher depressive symptoms and between frequent consumption of salad/raw vegetables and cooked vegetables and higher perceived stress among this group of female university students.

Keywords: perceived stress, depressive symptoms, university students, dietary behavior, mental health

INTRODUCTION

Stress and depression can result in a number of complications, including maladaptive eating behaviors such as overeating or undereating (Oliver et al., 2000; Kramlinger, 2001). These inconsistencies in dietary habits can be caused by stress and other factors, resulting in weight gain or loss in individuals with stress or depression. These changes in eating habits may eventually lead to changes in lifestyle behaviors (Schmidt, 2012). Additionally, undergoing stressful life situations could be a risk factor for developing depression (Kramlinger, 2001).

Over the past few years, the relationship between the type and the frequency of dietary intake has been studied in relation to mental health. This growing field of study concluded that higher consumption of processed and Western food indicates a higher risk of developing poor mental health (Rucklidge and Kaplan, 2016). Prospective studies have relied on causality to explain the nature of this relationship; unhealthy diets at baseline showed a higher risk of depression and poor mental health, whereas healthier diets at baseline showed better mental health and a lower risk of depression (Akbaraly et al., 2009; Jacka et al., 2011; Sánchez-Villegas et al., 2012). In experimental studies, when participants were exposed to a stressful situation, they tended to consume more of high-density foods and sweets (Oliver et al., 2000; Zellner et al., 2006). In addition, cross-sectional studies have suggested that higher perceived stress is positively associated with higher intake of fat, sodium, and carbohydrates (Nastaskin and Fiocco, 2015; Dehghan et al., 2016). However, other studies found that higher levels of stress were not associated with more sweets consumption but it was associated with lower fruit and vegetables intake (El Ansari and Berg-Beckhoff, 2015).

Moreover, systematic reviews have shown that foods rich in antidepressant nutrients, such as folate, iron, and chain omega-3 fatty acids (EPA and DHA), can enhance a person's mental health, and that people who consume these foods more often are less likely to develop depression (LaChance and Ramsey, 2018). The biological link between healthy diets and better mental health can be explained by several factors, including the neurotransmitter precursors, inositol and tryptophan (Grases et al., 2019). People with a higher depression score reported a lower consumption of legumes, fruits and vegetables which are among the foods that contain precursors to inositol and tryptophan which are important to brain health as opposed to people with lower scores (Grases et al., 2019). In line with these findings, cardioprotective diets that are high in fruits and vegetables and low in fat and refined sugar have been found to predict a lower risk of depression (Martínez-González, 2016). Overall, studies examining food choices under stress have found that people, particularly women, consume more types of food than they usually avoid for health reasons (Zellner et al., 2006). Regarding depression and dietary habits, most studies focused on the effects of certain diets on depression. These studies have shown that adherence to certain diets that provide healthier food choices results in improved mental health in depressed individuals (Jacka et al., 2017). Evidence also suggests that certain nutrients can positively affect mood and be used to treat depression, whereas other nutrients can increase the risk of it (Volker and Ng, 2006; Popa and Ladea, 2012). Moreover, it has been reported that poor dietary patterns could promote depressive symptoms (Jacka et al., 2014).

Studies that have sought to map the relationship between mental health symptoms (stress and depressive symptoms) and diet have reported that unhealthy food intake increases with perceived stress and depressive symptoms. Furthermore, unhealthy food consumption was linked to perceived stress in women only; however, depressive symptoms were linked to unhealthy food intake in both males and females (Mikolajczyk et al., 2009; El Ansari et al., 2014). A similar study concluded that levels of perceived stress and depressive symptoms increased with a decrease in fresh food intake and an increase in ready-to-eat food intake, and vice versa (Liu et al., 2007).

College students are vulnerable to vicious cycles in which poor diet choices and mental health symptoms perpetuate each other. Due to their new environment, responsibilities, financial pressure, and time management struggles, students are more prone to experience stress (Schmidt, 2012). Long-lasting stress has been found to be associated with weight gain as it triggers eating more frequently and less healthy (Torres and Nowson, 2007; Roberts, 2008). Depression can also lead to overeating or undereating (Kramlinger, 2001; Volker and Ng, 2006). However, research on the effects of stress and depression on dietary habits and choices in the UAE or the Middle East, specifically in Gulf Cooperation Council (GCC) countries, is limited and outdated. To the best of our knowledge, no studies have been conducted on UAE college students regarding the relationships between dietary habits, depressive symptoms, and perceived stress. Therefore, in this study, the association between frequency of food consumption and two mental health indicators (perceived stress and depressive symptoms) was assessed in female university students in the UAE.

MATERIALS AND METHODS

Study Design

This cross-sectional study was conducted following obtaining ethical clearance. After obtaining written informed consent, the participants were asked to complete a 12-item food frequency questionnaire (FFQ), Beck Depression Inventory, and Cohen's Perceived Stress Scale, which were compiled into a single questionnaire. The details of the study methodology have been described elsewhere (Ali et al., 2021).

Population and Sampling

The sample was drawn from female students at a national university in the United Arab Emirates (UAE). Several classes were randomly chosen from a list of courses held in spring 2018. The data were collected from April to May 2018. The sample represented ∼10% of the target population of 4,000 students and resulting in a total sample size of 389 students. A total of 385 questionnaires were accepted, resulting in a response rate of 98.9%. Before data collection, a pilot study was conducted on a sample of ten students to verify that the questionnaire was well-understood and appropriate for use with the intended sample. Pilot study participants were not included in the final data analysis.

Materials

Data were collected through a self-reported questionnaire consisting of three sections: a 12-item food frequency questionnaire, the 10-item Cohen's Perceived Stress Scale (PSS), and the Beck Depression Inventory-II (BDI-II). Weight and height were self-reported, which might have been a potential source of bias.

Food Frequency Questionnaire

The Food Frequency Questionnaire (Mikolajczyk et al., 2009) assessed the frequency of consumption of 12 food groups: sweets, cake/cookies, snacks, fast food/canned food, fresh fruits, salad/raw vegetables, cooked vegetables, soft drinks, meat/sausage products, fish/seafood, milk/milk products, and cereal/cereal products. Students were asked to answer the question "How often do you eat the following foods?" on a 5-point scale (several times a day = 5, daily = 4, several times a week = 3, 1–4 times a month = 2, and never = 1). Cronbach's α = 0.68. The FFQ was used in previous studies (Mikolajczyk et al., 2009; El Ansari et al., 2014; El Ansari and Berg-Beckhoff, 2015). Although not formally validated, the FFQ we used contains food groups that are essential for studying dietary behavior, which in line with the contents of other validated FFQs (Mikolajczyk et al., 2009).

Cohen's Perceived Stress Scale

The PSS estimates the degree to which situations in a person's life are considered stressful through 10 items (Cohen et al., 1983; Cohen and Williamson, 1988). Students expressed their feelings and thoughts for each item during the past month on a 5-point scale (0 = Never, 1 = Almost never, 2 = Sometimes, 3 = Fairly often, and 4 = Very often). In this study, the Cronbach's α was 0.68.

Beck Depression Inventory-II

The BDI-II measures the behavioral manifestation of depression (Salkind, 1969; Beck et al., 1996). The BDI-II was created in 1996 in alignment with the DSM-IV's diagnostic criteria for major depressive episode (Wang and Gorenstein, 2013). The BDI-II consists of 21 items with four statements each. Students were asked to choose one statement for each item that best described their feelings over the previous two weeks. The statements were scored on a 4-point scale ranging from 0 to 3. The creators of the BDI-II have enhanced validity compared to the original BDI (Beck et al., 1996). The Arabic version of the BDI-II (Ghareeb, 2000) was validated in 18 Arab countries: Palestine, Lebanon, Syria, Jordan, Saudi Arabia, Kuwait, Qatar, Bahrain, the United Arab Emirates, Oman, Yemen, Egypt, Sudan, Tunisia, Libya, Algeria, and Morocco, with Cronbach's α falling between 0.82 to 0.93 (Alansari, 2006; Maamria, 2010). To compensate for the removal of item 9 (suicidal thoughts and wishes), the scoring system was adjusted according to the ethical committee's instructions.

As a result, the BDI-II cut-off points were converted to percentages. The original BDI-II had a total score of 63, and the new score after deleting item 9 was 60. The BDI-II percentages were calculated by dividing by 63 and multiplying by 100 (63 was the total of score). The modified version of the BDI-II consists of 20 items, with a Cronbach's α of 0.89.

The original cut-off points of BDI-II:	The cut-off points as percentages:
(1–10) Normal ups and downs	(1.59–15.85) Normal ups and downs
(11–16) Mild mood disturbances	(17.46–25.40) Mild mood disturbances
(17–20) Borderline clinical depression	(27.98–31.75) Borderline clinical depression
(21–30) Moderate depression	(33.33–47.62) Moderate depression
(31–40) Sever depression	(49.21–63.49) Severe depression
(Over 40) Extreme depression	(Over 65.08) Extreme depression

Main Variables and Statistical Analysis

The FFQ consisted of 12 items, and was measured on a five-point scale (several times a day = 5, daily = 4, several times a week = 3, 1–4 times a month = 2, and never = 1).

To compare the results with those of other studies (Mikolajczyk et al., 2009), and by considering the least moderate correlations (Spearman > 0.2) among the items of each subscale and factor analysis and based on theoretical considerations regarding the content of foods, it was decided to combine some food groups into subscales. The following subscales were used: unhealthy foods subscale that consisting of sweets, cakes/cookies, snacks, and fast food and a fresh food subscale, consisting of fresh fruits, salads/raw vegetables, and cooked vegetables. The remaining food groups were divided into separate subscales. The subscales' scores were calculated as mean scores of the corresponding items.

The BDI-II score (BDI-II sum) was computed by summing the responses to all the 20 items that measured this mental health indicator. The Perceived Stress Score variable (PSS sum) was generated by summing all responses to all items of Cohen's Perceived Stress Scale after reversing scores to the four positively stated items (statements 4, 5, 7, and 8). Reversing was performed by recoding four statements: 0 for 4, 1 for 3, 2 kept as 2, 3 for 1, and 0 for 4.

The normality of all score variables was checked using kurtosis and skewedness, histograms, and Q_Q plots. All variables were normally distributed.

Independent associations between food groups (FFQ), perceived stress (PSS) and depressive symptoms (BDI-II) were examined using univariate and multivariate regression models. The newly created FFQ sum, PSS sum, and BDI-II sum served as dependent variables and were used for the subsequent data analysis. Sociodemographic and anthropometric variables were used as independent variables.

The collected data were coded, entered, and analyzed using the Statistical Package SPSS version 25. Statistical tests with *p*-values < 0.05 were considered statistically significant. Descriptive statistics was computed to describe all questionnaire items.

TABLE 1 | Sociodemographic characteristics and weight status (n = 385).

Characteristics	n	%
Emirate		
Dubai	290	75.3
Sharjah	49	12.7
Other	46	12.0
Marital Status		
Single	355	92.4
Married	29	7.6
Age		
17–20 years	233	62.2
21–24 years	134	35.7
25–29 years	8	2.1
Nationality		
Emirati	374	97.1
Non-emirati	11	2.9
University Year		
First year	87	23.0
Second year	90	23.8
Third year	113	29.9
Fourth years	88	23.3
BMI		
Underweight	49	13.4
Normal	196	53.6
Overweight	63	17.2
Obese	58	15.8

Multiple linear regression analysis was performed to predict each outcome variable using the following predictors: Marital status, age, BMI, exercise, stress, and depressive symptoms.

RESULTS

Sociodemographic Characteristics

Table 1 shows the participants' sociodemographic characteristics. The study population mainly consisted of Emiratis (97.1%). Their ages ranged from 17 to 24 years old. Regarding self-reported height and weight, 12.7% of the participants were underweight, 16.4% were overweight, and 15.1% were obese.

The mean scores were computed for the unhealthy food and fresh food subscales. Among this group of students, both unhealthy food and fresh food subscales had an average consumption (on a scale from 1 to 5). Overall, this group of students fell under the categories of "mild mood disturbances" and "moderate stress" for depressive symptoms and stress respectively.

Association Between Food Intake and Perceived Stress or Depressive Symptoms

The associations between the consumption of each food group and perceived stress and depression were assessed separately using a linear regression. **Table 2** shows that the significant associations between food groups, perceived stress, and depressive symptoms were not equal, whereas there were four associations for perceived stress and only three for depressive symptoms. Furthermore, negative associations were observed between perceived stress and cake/cookies and meat/sausage products, and between depressive symptoms and fresh fruits. Additionally, positive associations were observed between perceived stress and salad/raw vegetables and cooked vegetables and between depressive symptoms and soft drinks and fast food/canned food.

TABLE 2 | Associations between food intake and perceived stress or depressive symptoms.

Food group	PSS[a]		BDI-II[b]	
	P-Value	Estimate*	*P*-Value	Estimate*
Sweets	0.369	−0.046	0.517	0.033
Cake/cookies	**0.039**	−0.106	0.296	−0.054
Snacks	0.417	−0.042	0.534	0.032
Fast food/canned food	0.333	−0.049	**0.025**	0.114
Fresh fruits	0.059	−0.097	**0.017**	−0.122
Salad/raw vegetables	**0.045**	0.103	0.209	0.064
Cooked vegetables	**0.038**	0.107	0.096	0.086
Soft drinks	0.697	0.020	**0.015**	0.124
Meat/sausage products	**0.011**	−0.130	0.159	−0.072
Fish/sea food	0.055	−0.098	0.322	−0.051
Milk/milk products	0.324	−0.051	0.951	−0.003
Cereal/cereal products	0.251	−0.059	0.984	−0.001
Unhealthy Food Subscale	0.127	−0.078	0.377	0.045
Fresh Food Subscale	0.295	0.054	0.685	0.021

Estimates are the Standardized Coefficients. Change in food consumption for every one-unit change in the PSS or BDI-II.
[a]*PSS, Perceived Stress Scale.*
[b]*BDI-II, Beck Depression Inventory II.*
Food groups and PSS and BDI-II (univariable analysis). Bold means significant difference.

For every unit increase in PSS, the consumption of cake/cookies will decrease by 0.106, and meat/sausage product consumption will decrease by 0.130. However, salad/raw vegetable consumption will increase by 0.103 and cooked vegetable consumption will increase by 0.107. Regarding depressive symptoms, for every unit increase in the BDI-II, fresh fruits consumption will decrease by 0.122, fast/canned food consumption will increase by 0.114, while soft drink consumption will increase by 0.124 (**Table 2**).

Multiple linear regressions were used to assess the association between the consumption of all food groups together and both the PSS and BDI-II (**Table 3**). It was found that the consumption of unhealthy food, fresh food, fish/seafood, milk/milk products, and cereal/cereal products was not significantly associated with perceived stress and depressive symptoms among this group of university students. In addition, increased consumption of soft drinks was significantly associated with higher depressive symptoms, whereas increased consumption of meat/sausage products was significantly associated with lower perceived stress.

TABLE 3 | Food groups, PSS and BDI-II (multivariable analysis).

Food Subscale	PSS[a]		BDI-II[b]	
	P-Value	Estimate*	*P*-Value	Estimate*
Unhealthy food Subscale**	0.129	−0.086	0.928	0.005
Fresh food Subscale***	0.087	0.093	0.364	0.049
Soft drinks	0.147	0.083	**0.012**	0.145
Meat/sausage products	**0.048**	−0.114	0.126	−0.088
Fish/sea food	0.146	−0.085	0.279	−0.063
Milk/milk products	0.819	0.013	0.668	0.025
Cereal/cereal products	0.565	−0.032	0.810	0.014

**Estimates are the Standardized Coefficients. Change in food consumption for every one-unit change in the PSS or BDI-II.*
***Unhealthy food Subscale: mean of four items (sweets, cakes/cookies, snacks, fast food).*
****Fresh Subscale: mean of three items (fresh fruits, salads, cooked vegetables). Bold means significant difference.*

TABLE 4 | General linear model for unhealthy food consumption.

Variable	β	S.E.[a]	*P*-value	95% Confidence Interval for β	
				Lower bound	Upper bound
Corrected model	-	–	0.141	-	-
Intercept	3.392	0.424	0.000	2.558	4.226
Marital Status	0.103	0.137	0.451	−0.166	0.373
Students Age	−0.011	0.021	0.599	−0.051	0.030
BMI	−0.011	0.041	0.793	−0.091	0.070
Exercise	−0.040	0.019	**0.042**	−0.078	−0.002
BDI-II	0.007	0.005	0.167	−0.003	0.016
PSS	−0.015	0.009	0.085	−0.032	0.002

R^2 = 0.028.
[a]S.E., standard error. Bold means significant difference.

Unhealthy Food Consumption

Table 4 presents the results of the multiple linear regression model of unhealthy food consumption. This included marital status, age, BMI, exercise, BDI-II sum, and PSS sum. These variables accounted for only ∼3% of the variation in the unhealthy food consumption subscale ($R^2 = 0.028$). However, this difference was not statistically significant ($p = 0.141$). Exercise was the only variable that had a significant effect on the unhealthy food consumption ($p < 0.050$).

DISCUSSION

The main objective of this study was to assess the association between perceived stress and depressive symptoms with the frequency of consumption of specific food groups among female university students. Habits formed during youth are likely to be sustained throughout later adulthood (Schmidt, 2012). Therefore, studying the complex relationship between food choice and mental state could help us to understand how the frequency of consumption of certain food groups could be influenced by stress or depression, or vice versa.

The results of this study showed that the consumption of cakes/cookies and meat/sausage products was negatively associated with stress, whereas consumption of salad/raw vegetables and cooked vegetables was positively associated with stress. These results are inconsistent with those of other cross-sectional studies conducted on different populations. Several studies have reported higher consumption of sweets, snacks, and fast food with higher stress levels or higher consumption of fruits and vegetables with lower stress levels (Mikolajczyk et al., 2009; El Ansari and Berg-Beckhoff, 2015). Nevertheless, a study conducted in Palestine reported results similar to those of the present study.

It was found that among females, the consumption of cakes/cookies was negatively associated with stress, and among males, all food groups except cereal/cereal products were negatively associated with stress (Mohamed Yassin, 2016). The latter study suggested that lower consumption of cakes/cookies with higher stress could be linked to the fact that people consume foods high in carbohydrates (CHO) to relieve their stress. This could be explained by the fact that highly palatable foods that are high in CHO and fat, such as cakes/cookies, enhance opioid levels in the brain, which are linked to feelings of pleasure and are eaten as rewards after stress. Hence, students' stress levels were lower when they consumed these foods (Society for the Study of Ingestive Behavior, 2009; Mohamed Yassin, 2016). However, this suggestion is based on the assumption that students with lower stress levels who consumed more cakes and cookies were stressed before, and consumed more of this food group to relieve their symptoms. This assumption could not be confirmed by the current study design. Another possible explanation could be behavioral differences between stress and depression among individuals.

This study found that the consumption of fast food/canned food and soft drinks was positively associated with depressive symptoms whereas, the consumption of fresh fruits was negatively associated with depressive symptoms. These results are consistent with those of similar studies found regarding these food groups and depressive symptoms (Mikolajczyk et al., 2009; El Ansari et al., 2014; Martínez-González, 2016). Previous studies have found that consuming foods such as fruits, vegetables, seafood, and low processed organ meats that are rich in antidepressant nutrients is associated with a lower risk of depression (Martínez-González, 2016). In addition to the fact that highly processed and high-sugar foods are among the foods linked to depression, low-processed organ meats, seafood, and legumes were found to have the highest levels of antidepressants (Martínez-González, 2016). In line with these findings, the current study found a negative association between vegetable consumption and depression; however, no significant effects were found regarding for seafood, organ meat, or legumes consumption. Depression reflects behavioral changes in people with higher levels of depressive symptoms. Thus, individuals with depressive symptoms are more likely isolate themselves from their social networks, which might increase their symptoms. Unlike stress, depression creates feelings of emptiness and hopelessness, pointlessness of efforts and severe reduction in the perception of self-efficacy (Brouwers and Tomic, 2000; Bisschop et al., 2004; Gallagher et al., 2011).

As such, making an effort to eat healthier food for people with depression would not be significant; hence, the tendency we report here to consume more easily accessible foods that fall under the unhealthy food groups in the high-depression group. However, it may still be considered important and possible to eat healthily; hence, there is a positive link between higher levels of stress and healthier eating behaviors. It may be interest in the future to investigate the extent to which there is a discrepancy between the wish to eat healthily and the actual dietary behavior of the high-stress group. Importantly, it would be interesting to determine the extent to which a higher discrepancy (between wish and actual dietary behavior) is linked via dissonance, to the levels of stress reported in this group.

This study found only one significant association between stress and one with depressive symptoms. Consumption of meat/sausage products was negatively associated with stress, whereas the consumption of soft drinks was positively associated with depressive symptoms. These findings are not consistent with findings from other studies that found positive associations between unhealthy food (sweets/snacks/cookies/fast food) and stress and depression, nor with studies that found negative associations between healthy food (fruits and vegetables) and stress and depression (Mikolajczyk et al., 2009; El Ansari et al., 2014). However, Yassin et al. found that stress was negatively associated with unhealthy food consumption among males only, which is similar to the insignificant result found in this study regarding the unhealthy food subscale and stress (Mohamed Yassin, 2016). First, the explanation for the negative association found between meat/sausage product consumption and stress is not known, although it could mean that lower stress leads to an increased consumption of protein. Future studies are needed to further investigate this association. Second, the positive association between soft drink consumption and depressive symptoms could be related to behavioral changes in people with higher depressive symptoms, as previously mentioned.

Finally, the general linear regression model controlling for confounding variables showed that exercise was the only variable that predicted unhealthy food consumption. The results showed that the frequency of exercise decreased with a higher consumption of unhealthy food. Previous studies found that individuals who report higher levels of physical activity have lower levels of stress and depression. Therefore, there may be an indirect association between the two mental health indicators and unhealthy food consumption (Norris et al., 1992; Hassmén et al., 2000). Unhealthy food consumption could be affected more by variables not included in this study, which should be investigated in future studies. The chief among these are socioeconomic variables, variables related to parents' educational level, and other social factors. As this study is cross-sectional, causation could not be derived and was susceptible to bias. Therefore, future studies should focus on clinical and controlled experiments.

CONCLUSION

This study found associations between stress, and depression, with food consumption among female university students. In contrast to stress, feelings of hopelessness, which are symptomatic of depression, reduce the importance of consuming healthier food. Additionally, eating cakes/cookies was negatively associated with perceived stress. However, assessing the presence of pre-existing stress that affects food consumption was not within the scope of this study. Further studies are required to investigate the relationship between stress and food consumption in this population and to identify the main reasons for this relationship. This study suggests identifying nutritional issues when addressing perceived stress or depressive symptoms among female university students. Moreover, improving the mental state of students could improve their food choice and intake.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mercedes Sheen Chair of the Research Ethics Committee, Zayed University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

OM wrote the initial draft of the manuscript, compiled the instruments in one questionnaire and performed data collection. HA supervised the project, provided guidance and advice when needed. LS performed data analysis, wrote the initial draft of the statistical analysis and results. EH provided guidance regarding the mental health indicators and the instruments used to assess them in addition to contributing to the discussion. RA provided guidance regarding the food frequency questionnaire and suggested the adjustments of the BDI-II scoring system to compensate for the obligatory removal of one item. All authors contributed to the article and approved the submitted version.

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REFERENCES

Akbaraly, T. N., Brunner, E. J., Ferrie, J. E., Marmot, M. G., Kivimaki, M., and Singh-Manoux, A. (2009). Dietary pattern and depressive symptoms in middle age. *Br. J. Psychiatry* 195, 408–413. doi: 10.1192/bjp.bp.108.058925

Alansari, B. M. (2006). Internal consistency of an Arabic adaptation of the beck depression inventory-ii with college students in eighteen Arab countries. *Soc. Behav. Pers.* 34, 25–30. doi: 10.2224/sbp.2006.34.4.425

Ali, A., Hendawy, A., Ahmad, O., Al Sabbah, H., Smail, L., and Kunugi, H. (2021). The Arabic version of the cohen perceived stress scale: factorial validity and measurement invariance. *Brain Sci*. 11, 419. doi: 10.3390/brainsci11040419

Beck, A. T., Steer, R. A., and Brown, G. K. (1996). *Manual for the Beck Depression Inventory-II, 2nd Edn*. San Antonio, TX: Psychological Corporation.

Bisschop, M. I., Kriegsman, D. M. W., Beekman, A. T. F., and Deeg, D. J. H. (2004). Chronic diseases and depression: the modifying role of psychosocial resources. *Soc. Sci. Med*. 59, 721–733. doi: 10.1016/j.socscimed.2003.11.038

Brouwers, A., and Tomic, W. (2000). A longitudinal study of teacher burnout and perceived self-efficacy in classroom management. *Teach. Teach. Educ*. 16, 239–253. doi: 10.1016/S0742-051X(99)00057-8

Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *J. Health Soc. Behav*. 24, 385–396. doi: 10.2307/2136404

Cohen, S., and Williamson, G. (1988). "Perceived stress in a probability sample of the United States," in *The Social Psychology of Health*, eds S. Spacapan and S. Oskamp (Newbury Park, CA: SAGE), 31–67.

Dehghan, P., Pourmoradian, S., Mahdavi, A. M., Sarmadi, B., and Mehralizadeh, S. (2016). Relationship between perceived stress and dietary intakes in type 2 diabetic patients. *Curr. Top. Nutraceuticals Res*. 14, 199–206. Available online at: https://www.researchgate.net/publication/309557097_RELATIONSHIP_BETWEEN_PERCEIVED_STRESS_AND_DIETARY_INTAKES_IN_TYPE_2_DIABETIC_PATIENTS

El Ansari, W., Adetunji, H., and Oskrochi, R. (2014). Food and mental health: relationship between food and perceived stress and depressive symptoms among university students in the United Kingdom. *Cent. Eur. J. Public Health*. 22, 90–97. doi: 10.21101/cejph.a3941

El Ansari, W., and Berg-Beckhoff, G. (2015). Nutritional correlates of perceived stress among university students in Egypt. *Int. J. Environ. Res. Public Health*. 12, 14164–14176. doi: 10.3390/ijerph121114164

Gallagher, D., Mhaolain, A. N., Crosby, L., Ryan, D., Lacey, L., and Coen, R. F. (2011). Self-efficacy for managing dementia may protect against burden and depression in Alzheimer's caregivers. *Aging Ment Heal*. 15, 663–670. doi: 10.1080/13607863.2011.562179

Ghareeb, G. A. (2000). *Beck Depression Inventory-II Studies of Instructions, Studies of Reliability and Validity, Standardization and Cut-Off Points*. Cairo: The Anglo Egyptian Bookshop.

Grases, G., Colom, M. A., and Sanchis, P. (2019). Possible relation between consumption of different food groups and depression. *BMC Psychol.* 7, 14. doi: 10.1186./s40359-019-0292-1

Hassmén, P., Koivula, N., and Uutela, A. (2000). Physical exercise and psychological well-being: a population study in Finland. *Prev Med (Baltim)*. 30, 17–25. doi: 10.1006/pmed.1999.0597

Jacka, F. N., Cherbuin, N., Anstey, K. J., and Butterworth, P. (2014). Dietary patterns and depressive symptoms over time: examining the relationships with socioeconomic position, health behaviours and cardiovascular risk. *PLoS ONE* 9, e87657. doi: 10.1371/journal.pone.0087657

Jacka, F. N., Kremer, P. J., Berk, M., de Silva-Sanigorski, A. M., Moodie, M., Leslie, E. R., et al. (2011). A prospective study of diet quality and mental health in adolescents. *PLoS ONE* 6, e24805. doi: 10.1371/journal.pone.0024805

Jacka, F. N., O'Neil, A., Opie, R., Itsiopoulos, C., Cotton, S., and Mohebbi, M. (2017). A randomised controlled trial of dietary improvement for adults with major depression (the "SMILES" trial). *BMC Med.* 15, 1–13. doi: 10.1186/s12916-017-0791-y

Kramlinger, K. (2001). *Mayo Clinic on Depression: Answers to Help You Understand, Recognize and Manage Depression*. Rochester, MN: Mayo Clinic.

LaChance, L. R., and Ramsey, D. (2018). Antidepressant foods: an evidence-based nutrient profiling system for depression. *World J Psychiatry* 8, 97–104. doi: 10.5498/wjp.v8.i3.97

Liu, C., Xie, B., Chou, C. P., Koprowski, C., Zhou, D., and Palmer, P. (2007). Perceived stress, depression and food consumption frequency in the college students of China seven cities. *Physiol. Behav*. 92, 748–754. doi: 10.1016/j.physbeh.2007.05.068

Maamria, B. (2010). Standardizing Beck Depression Inventory-II on samples from the two genders in the Algerian environment. *Arab Ejournal*. 25, 92–105. Available online at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3539842/pdf/JMedLife-05-414.pdf

Martínez-González, M. A. (2016). Food patterns and the prevention of depression. *Proc. Nutr. Soc.* 75, 139–146. doi: 10.1017/S0029665116000045

Mikolajczyk, R. T., El Ansari, W., and Maxwell, A. E. (2009). Food consumption frequency and perceived stress and depressive symptoms among students in three European countries. *Nutr. J.* 8, 1–8. doi: 10.1186/1475-2891-8-31

Mohamed Yassin, M. (2016). Food consumption in association with perceived stress and depressive symptoms: a cross sectional study from five universities and three colleges in Gaza Strip, Palestine. *Eur. J. Prev. Med*. 4, 20. doi: 10.11648/j.ejpm.20160401.14

Nastaskin, R. S., and Fiocco, A. J. (2015). A survey of diet self-efficacy and food intake in students with high and low perceived stress. *Nutr. J.* 14, 42. doi: 10.1186/s12937-015-0026-z

Norris, R., Carroll, D., and Cochrane, R. (1992). The effects of physical activity and exercise training on psychological stress and well-being in an adolescent population. *J Psychosom Res*. 36, 55–65. doi: 10.1016/0022-3999(92)90114-H

Oliver, G., Wardle, J., and Gibson, E. L. (2000). Stress and food choice: A laboratory study. *Psychosom. Med*. 62, 853–865. doi: 10.1097/00006842-200011000-00016

Popa, T. A., and Ladea, M. (2012). Nutrition and depression at the forefront of progress. *J. Med. Life*. 5, 414–419. Available online at: http://arabpsynet.com/Archives/OP/eJ25-26BachirMaamria.pdf

Roberts, C. J. (2008). The effects of stress on food choice, mood and bodyweight in healthy women. *Nutr. Bull*. 33, 33–39. doi: 10.1111/j.1467-3010.2007.00666.x

Rucklidge, J. J., and Kaplan, B. J. (2016). Nutrition and mental health. *Clin. Psychol. Sci*. 4, 1082–1084. doi: 10.1177/2167702616641050

Salkind, M. R. (1969). Beck depression inventory in general practice. *J. R. Coll. Gen. Pract*. 18, 267–271.

Sánchez-Villegas, A., Toledo, E., De Irala, J., Ruiz-Canela, M., Pla-Vidal, J., and Martínez-González, M. A. (2012). Fast-food and commercial baked goods consumption and the risk of depression. *Public Health Nutr*. 15, 424–432. doi: 10.1017/S1368980011001856

Schmidt, M. (2012). Predictors of self-rated health and lifestyle behaviours in Swedish university students. *Glob. J. Health Sci*. 4, 1-14. doi: 10.5539/gjhs.v4n4p1

Society for the Study of Ingestive Behavior (2009). High-fat, High-sugar Foods Alter Brain Receptors. *ScienceDaily*. Available online at: https://www.sciencedaily.com/releases/2009/07/090727102024.htm (accessed February 13, 2022).

Torres, S. J., and Nowson, C. A. (2007). Relationship between stress, eating behavior, and obesity. *Nutrition* 23, 887–894. doi: 10.1016/j.nut.2007.08.008

Volker, D., and Ng, J. (2006). Depression: does nutrition have an adjunctive treatment role? *Nutr. Diet*. 63, 213–226. doi: 10.1111/j.1747-0080.2006.00109.x

Wang, Y. P., and Gorenstein, C. (2013). Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. *Rev. Bras. Psiquiatr*. 35, 416–431. doi: 10.1590/1516-4446-2012-1048

Zellner, D. A., Loaiza, S., Gonzalez, Z., Pita, J., Morales, J., and Pecora, D. (2006). Food selection changes under stress. *Physiol. Behav*. 87, 789–793. doi: 10.1016/j.physbeh.2006.01.014

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Association of High Dietary Acid Load With the Risk of Cancer: A Systematic Review and Meta-Analysis of Observational Studies

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Objective: This study aimed to determine the relationship between the high dietary acid load (DAL) and the risk of cancer.

Methods: Five databases of PubMed, Web of Sciences, Scopus, Cochrane Library, and Google Scholar was searched to elicit original studies on humans, up to June 2021. Quality of the articles, risk of bias, and heterogeneity were assessed. A random-effects meta-analysis model was applied to estimate pooled effect size with a 95% confidence interval. Sensitivity analysis was performed using a fixed-effects model. Subgroup analyses were carried out based on gender, age, type of cancer, and type of DAL assessment indicator.

Results: Seventeen effect sizes from 10 articles were included in the analysis. Overall, individuals with the highest DAL were associated with a 66% increased risk of cancer compared to those with the lowest DAL ($p < 0.001$]. The risk of cancer increased 41% ($p < 0.001$) and 53% ($p = 0.03$) by high PRAL and NEAP, respectively. High DAL was associated with 32% ($p < 0.001$) and 79% ($p < 0.001$) increased risk of breast and colorectal cancers, respectively. High DAL was associated with 32% ($p = 0.001$) and 76% ($p = 0.007$) increased risk of cancer incident in women and men, respectively. The risk of cancer incident increased 35% ($p < 0.001$) and 49% ($p < 0.001$) at age $\leq$ and $>$ of 50, respectively.

Conclusion: High DAL may be associated with a higher risk of cancer incidence not only in the whole studied population but also across cancer types, both genders, both DAL assessment indicators, and also among both high- and low-risk age groups for cancer.

Keywords: dietary acid load, cancer, systematic review, meta-analysis, observational studies

INTRODUCTION

Cancer is a major burden of disease and health concern worldwide. It is the second leading cause of mortality in many countries (1) and accounting for around 10 million deaths in 2020 (2). It has been well known that lifestyle could influence the risk of cancer (3). An individual's diet is a major modifiable lifestyle-related factor that may be linked to his/her health outcomes. Numerous epidemiological investigations have indicated that diet composition or pattern can contribute to or prevent the development of chronic diseases including cancer (4–6). According to the previous studies, adherence to a plant-based diet with low animal and processed food products may prevent the risk of cancer (7, 8).

It has recently been suggested that diet composition may influence the body's acid-base balance (9). Some dietary components are acidogenic and increase the dietary acid load (DAL). Animal proteins and cereal grains are dietary components that are metabolized to acid precursors and generate acid in the body (10, 11). While, some food ingredients such as fruits and vegetables, due to containing potassium, or dairy products, due to consisting of calcium and magnesium, produce precursors of alkali and may reduce diet-dependent acid load (10, 11).

As the DAL correlates with the urinary acid load, it has been suggested as a simple and useful method to evaluate the acidity of a diet (12). Potential renal acid load (PRAL) and net endogenous acid production (NEAP) are two common established indicators to calculate metabolic acidosis and estimate the DAL from dietary intake data (13, 14). PRAL presents an assessment of the endogenous acid production that exceeds the alkali level produced for certain amounts of food consumed daily. Daily PRAL is a measure that considers the dietary composition of several minerals and proteins (particularly sulfur-containing proteins) and their mean intestinal absorption rate, and the amount of sulfate generated from metabolized proteins. PRAL is calculated using the following formula:

$$\text{PRAL (mEq/day)} = 0.4888 \times \text{protein intake (g/day)} + 0.0366 \times \text{phosphorus (mg/day)} - 0.0205 \times \text{potassium (mg/day)} - 0.0125 \times \text{calcium (mg/day)} - 0.0263 \times \text{magnesium (mg/day)}$$

NEAP is assessed from the ratio of protein and potassium in the diet and calculated using the following formula:

$$\text{NEAP (mEq/day)} = 54.5 \times \text{protein (g/day)}/\text{potassium (mEq/day)} - 10.2$$

It has been shown that greater intake of a diet with high acid load may contribute to the increased risk of health conditions such as cardiovascular diseases (15), hypertension (16), chronic kidney disease (17), and diabetes mellitus (18). Multiple investigations have recently studied the association between the DAL and the risk of various cancers (19–24). However, to our knowledge, there has been no comprehensive report summarizing these studies. Therefore, this systematic review and meta-analysis study was implemented to summarize the present studies in order to determine "What is the risk of cancer incidence in adults with high DAL compared to those with low DAL?"

METHODS

This systematic review and meta-analysis study follows the updated 2020 Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guidelines (25). The protocol of the study was registered and approved by the Ethical Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1400.560) and is available at: https://ethics.research.ac.ir/IR.TBZMED.REC.1400.560.

Search Strategy

An extensive systematic search of the literature was performed in electronic databases of PubMed, Web of Sciences, Scopus, Cochrane Library, and Google Scholar up to June 2021, with no publication date restriction. This was supplemented by searching for reference lists and citation tracking of included studies, and relevant reviews. The keywords and medical subject headings (MeSH) terms used for the search were as follows: "acid load OR dietary acid load OR potential renal acid load OR net endogenous acid production" AND "cancer." The full search method for each database is available in **Supplementary Table 1**.

The articles from the initial searches were imported into an EndNote software and duplicates were removed. Titles and abstracts of the remained articles were independently screened for potential eligibility by two reviewers (M.K and V.M) and any discrepancy was resolved by discussion or third researcher.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: studies that considered the association between DAL and cancer; studies with prospective or retrospective cohort and case-control design; studies that expressed odds ratios (ORs) or hazard ratios (HRs) or relative risks (RRs) beside 95% confidence intervals (CIs) for the association between DAL and cancer. Studies with cross-sectional design, letters, comments, short communications, surveys, environmental, and animal studies were excluded.

Data Extraction

The required data were extracted from each eligible study by two independent researchers and any disagreement between the two researchers was resolved by discussion or by a third researcher. The extracted information was as follows: Name of the first author, year of publication, country, study design, type of studied cancer, number of participants, mean age and gender of participants, follow-up time for cohort studies, method of food intake assessment, method of DAL assessment, confounding variables, outcomes, and information regarding OR or HR or RR and 95% CI. If a study used both of the PRAL and NEAP indicators for assessing DAL, we considered that study as two separate studies in meta-analysis.

Quality Assessment

The Newcastle Ottawa (NOS) scale (26) was used to evaluate the quality of the selected studies. Based on this scale, a maximum of 9 scores is allocated to each study as follows: four scores for selection of contributors, two scores for comparability, and three scores for evaluating outcomes in cohort studies and exposure in

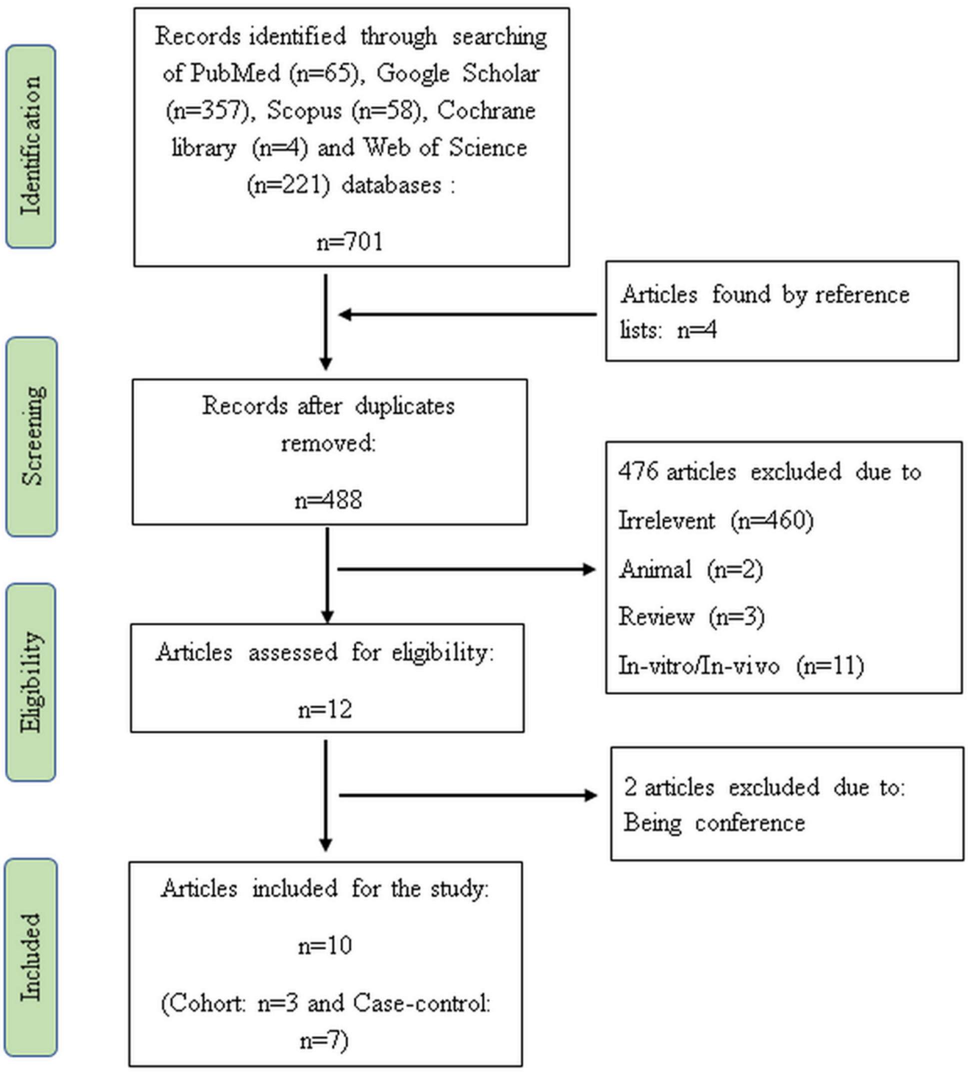

FIGURE 1 | Flow diagram of the study.

case-control studies. Studies attaining 9 scores were considered as the highest quality.

Statistical Analysis

A random-effects model was used to estimate the pooled effect size (d) for comparison of the highest vs. the lowest categories of the DAL and to consider the heterogeneity between the studies (27). The random-effects model was used to estimate the Q-statistics and I^2 values as heterogeneity indices. I^2 value > 50% between-study heterogeneity was considered significant. When between-study heterogeneity was significant, we performed subgroup analyses based on participants' gender, the mean age of the participants, type of cancer, and type of DAL assessment indicator to determine possible sources of heterogeneity. Publication bias was assessed using Egger's and Begg's regression asymmetry test (28). Small study bias, including publication bias, was detected by visually inspecting funnel plots. A trim-and-fill method was used to determine the effect of possible missed studies on the overall effect (29). Sensitivity analysis was performed using a fixed-effects model in which each study was eliminated from the study to evaluate the influence of that study.

STATA version 14.0 was applied to perform statistical analyses. A p-value < 0.05 was reflecting the statistical significance of all tests.

RESULTS

Literature Search

In the initial search, 705 articles were detected. After elimination of duplicates ($n = 217$), irrelevant ($n = 460$), animal ($n = 2$), review ($n = 3$), and *in-vitro*/*in-vivo* articles (n

TABLE 1 | Characteristics of the included studies.

References	Study design	Country	Type of cancer	Gender	Participants (case/control)	Mean age (year)	Follow-up time	Food intake assessment tool	DAL assessment indicators	Confounders considered in the analysis	Outcomes
Jafari et al. (19)	Case–control	Iran	Colorectal	Both	499 (259/240)	50	-	FFQ	PRAL	Age, comorbidity, CFH, salt intake, physical activity, and Ca supplement	PRAL↑ → risk of CRC and CRA↑
Mehranfar et al. (20)	Case–control	Iran	Prostate	Men	120 (60/60)	Not indicated	-	FFQ	PRAL & NEAP	Age, BMI, TEI, smoking, physical activity, race, job, education, and drug usage	PRAL↑ → risk of prostate cancer↑ NEAP↑ → risk of prostate cancer↑
Mousavi et al. (31)	Case–control	Iran	Glioma	Both	366 (123/243)	42	-	FFQ	NEAP	Age, sex, TEI, marital status, smoking, CFH, physical activity, supplementation, BMI, X-ray exposure, head trauma, allergy, duration of illness, micronutrient intake, and comorbidity	NEAP↑ → developing glioma among adults ↑
Park et al. (21)	Cohort	US and Puerto Rican	Breast	Women	43570	54.5	7.6 y	FFQ	PRAL	Race, education, household income, BMI, physical activity, smoking, alcohol, CFH, breastfeeding, TEI, and parity	PRAL↑ → risk of breast cancer ↑
Ronco et al. (22)	Case–control	Uruguay	Colorectal	Both	3005 (611/2394)	64	-	FFQ	PRAL & NEAP	Age, sex, residence, education, CFH, BMI, smoking, alcohol, TEI, total fiber, micronutrient, and total heterocyclic amines	PRAL ↑ → risk of colorectal cancer ↑ NEAP↑ → risk of colorectal cancer ↑
Ronco et al. (23)	Case–control	Uruguay	Lung	Men	2309 (843/1466)	65	-	FFQ	PRAL & NEAP	Age, residence, CFH, BMI, smoking, alcohol, TEI, total fiber, micronutrient, and total heterocyclic amines	PRAL↑ → was not significantly associated with lung cancer risk NEAP↑ → risk of lung cancer ↑
Ronco et al. (32)	Case–control	Uruguay	Breast	Women	1461 (572/889)	65	-	FFQ	PRAL & NEAP	Age, residence, education, age at menarche, menopausal status, number of live births, age at menopause, CFH, BMI, smoking, alcohol, and TEI	PRAL↑ → risk of breast cancer ↑ NEAP↑ → risk of breast cancer ↑
Safabakhsh et al. (33)	Case–control	Iran	Breast	Women	300 (150/150)	46.5	-	FFQ	PRAL & NEAP	Age at first menarche, BMI, education, marital status, menopause status, socioeconomic status, alcohol, smoking, supplementation, comorbidity, number of Child, breast feeding, CFH, and TEI	PRAL↑ → was not significantly associated with breast cancer risk NEAP↑ → was not significantly associated with breast cancer risk recurrence

(Continued)

TABLE 1 | Continued

Reference	Study design	Country	Type of cancer	Gender	Participants (case/control)	Mean age (year)	Follow-up time	Food intake assessment tool	DAL assessment indicators	Confounders considered in the analysis	Outcomes
Shi et al. (24)	Cohort	US	Pancreatic	Both	95708	64.5	8 y	FFQ & DHQ	PRAL	Age, sex, smoking, diabetes, alcohol, BMI, CFH, TEI, and dietary fiber	PRAL↑ → risk of pancreatic cancer ↑
Wu et al. (30)	Cohort	US	Breast	Women	2950	44	7.3 y	24-h food recall	PRAL & NEAP	Age at diagnosis, race, education, menopausal status at baseline, TEI, alcohol, physical activity, BMI, number of comorbidities, radiotherapy, and chemotherapy.	PRAL↑ → was not significantly associated with breast cancer recurrence NEAP↑ → was not significantly associated with breast cancer recurrence

DAL, dietary acid load; FFQ, food frequency questionnaire; DHQ, diet history questionnaire; PRAL, potential renal acid load; NEAP, net endogenous acid production; CFH, cancer family history; CRC, colorectal cancer; CRA, colorectal Adenoma; TEI, total energy intake.

= 11), 12 publications met the topic and scope of the study during the first screening phase. Two studies were also removed during the second screening phase because these studies were conference. Finally, three cohorts (21, 24, 30) and seven case-control studies (19, 20, 22, 23, 31–33) were comprised in the current systematic review and meta-analysis. **Figure 1** shows the flow diagram of the study selection process.

Characteristics of the Included Studies

Table 1 displays the characteristics of all the included studies. The total number of participants in three cohort studies was 142,228, and in seven case-control studies were 8,060 (2,618 patients with cancer and 5,442 controls). The follow-up period in cohort studies ranged from 7.3 to 8 years. The type of studied cancers were breast (21, 30, 32, 33), colorectal (19, 22), prostate (20), lung (23), pancreas (24), and glioma (31). Most of the studies ($n = 9$) used the food frequency questionnaire (FFQ) tool for assessment of the food intake. In most the papers, effect sizes were adjusted for age ($n = 10$), BMI ($n = 9$), sex ($n = 3$), smoking ($n = 8$), alcohol consumption ($n = 7$), physical activity ($n = 3$), energy intake ($n = 9$), comorbidities ($n = 4$), cancer family history ($n = 8$), menopausal status ($n = 4$), education ($n = 6$), residence ($n = 3$), race ($n = 3$), and other dietary variables ($n = 6$). DAL assessment indicator was PRAL in nine studies and NEAP in seven studies. All cohort and four case-control studies obtained the NOS score of 9 and were of high quality and the score of other studies were 8 (**Supplementary Tables 2, 3**).

Risk of Bias Assessment

The methodological characteristics of the included studies are summarized in **Supplementary Tables 2, 3**. In all of the articles, the most important confounders were controlled in the statistical analysis. In all studies, the selection of controls was done correctly, and in all studies, food intake was assessed by a structured interview.

Results of Systematic Review and Meta-Analysis

Results of the systematic review showed that of nine studies that investigated the relationship between PRAL and risk of cancer, six studies indicated a positive association. Five out of seven studies found a positive association between NEAP and cancer risk.

Seventeen effect sizes from 10 studies were included in this analysis. Comparing the highest against the lowest DAL, the pooled effect size for the risk of overall cancer was 1.66 (95% CI: 1.38, 2.01; $p < 0.001$), demonstrating a significant positive relationship (**Figure 2**). A significant heterogeneity between studies was observed ($I^2 = 72.0\%$; $p < 0.001$). As shown in **Figure 3**, results of subgroup analyses showed that gender, age of the participants, type of cancer, and type of DAL assessment indicator had not any role in the between-study heterogeneity.

As shown in **Figure 3**, according to stratified analysis, the risk of cancer incidence increased by 41% [d = 1.41 (95% CI: 1.27,

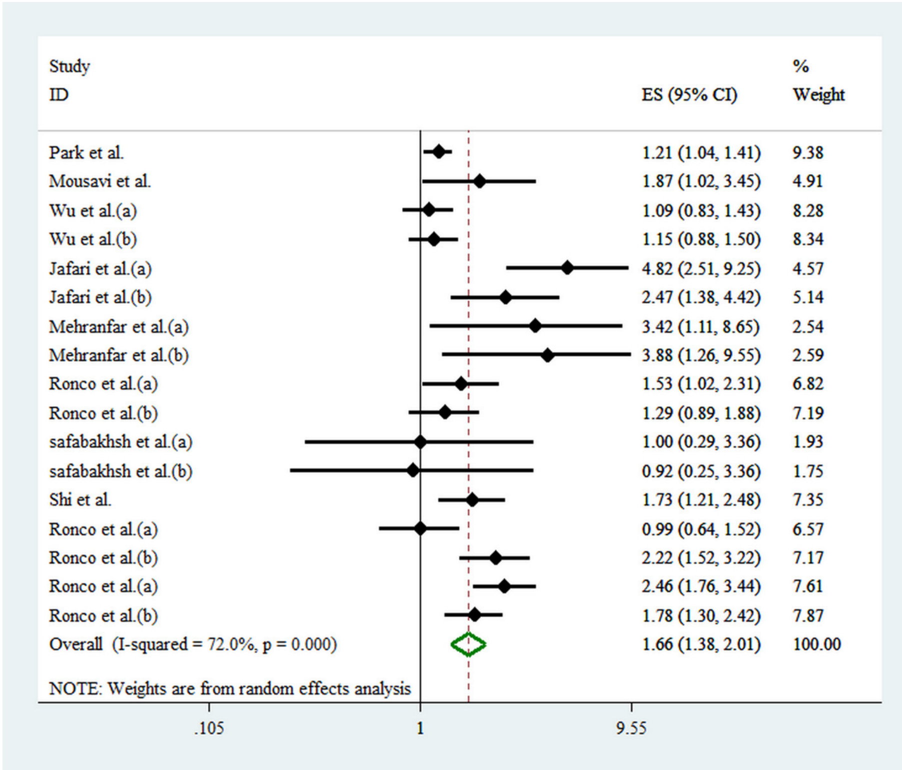

FIGURE 2 | Forest plot for the association between DAL and risk of cancer in a random-effects meta-analysis. ES, effect size; CI, confidence interval.

1.57), $p < 0.001$] and 53% [d = 1.53 (95% CI: 1.32, 1.79), $p = 0.03$] by high PRAL and NEAP, respectively. High DAL was associated with 32% [d = 1.32 (95% CI: 1.19, 1.46), $p < 0.001$] and 79% [d = 1.79 (95% CI: 1.42, 2.26), $p < 0.001$] increased risk of breast and colorectal cancers occurrence, respectively. High DAL was associated with 32% [d = 1.32 (95% CI: 1.19, 1.46), $p = 0.001$] and 76% [d = 1.76 (95% CI: 1.35, 2.29), $p = 0.007$] increased risk of cancer incidence in women and men, respectively. The risk of cancer incidence increased by 35% [d = 1.35 (95% CI: 1.15, 1.60), $p < 0.001$] and 49% [d = 1.49 (95% CI: 1.35, 1.65), $p < 0.001$] among people with age ≤ and > of 50, respectively.

Sensitivity Analyses and Publication Bias

Sensitivity analysis showed that the overall effect size regarding the association between DAL and cancer did not depend on a single study (95% CI: 1.32-2.13). Based on the visual inspection of the funnel plot, we found an asymmetry (**Supplementary Figure 4**); however, when we did the Egger's and Begg's regression test indicated possible publication bias for the association between DAL and cancer ($p = 0.038$). Therefore, we did the trim-and-fill method and found that adding missing studies did not change the overall effect size [d = 0.36 (95% CI: 0.16-0.56)] (**Supplementary Figure 2**).

DISCUSSION

In the present meta-analysis of observational studies, a significant association was observed between higher DAL and the risk of cancer occurrence in the entire population. We found that the risk of cancer increased by 66% in participants with higher DAL compared to the participants with lower DAL. The positive association remained significant across cancer types, both genders, both DAL assessment indicators (PRAL and NEAP), and also among both high- and low-risk age groups for cancer.

It is well known that factors such as sex, age, obesity, energy intake, smoking status, and physical activity level have a role in cancer development. On the other hand, the association of these factors with DAL has been evidenced in numerous researches. It has been shown that DAL has larger effects in the elderly than younger individuals and in women compared with men (34) and elder individuals may be more sensitive to DAL effects compared with younger persons (9). Fatahi et al. showed that the odds of general and abdominal adiposity increased across tertiles of DAL (35). Li et al. have also reported a positive association between high DAL and obesity in the nationally-representative sample of Chinese adults (36). Fatahi et al. in a study on women found a positive association between DAL and energy density (35). Kataya et al. in a study on elderly Japanese

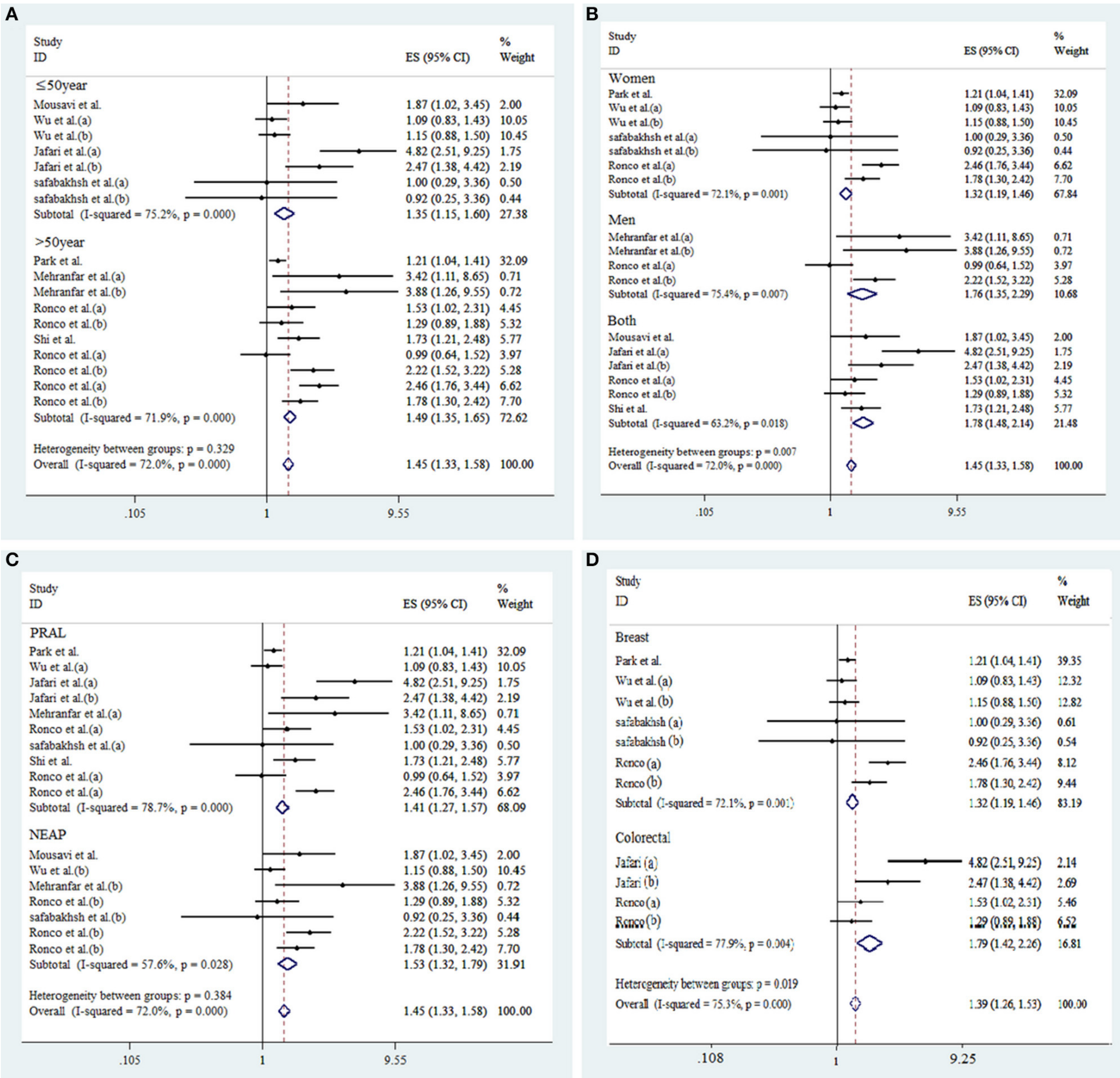

FIGURE 3 | Subgroup analysis for the association between DAL and risk of cancer. Subgroup by age of participants **(A)**, gender **(B)**, type of DAL assessment indicators **(C)**, and type of cancer **(D)**.

women found that high DAL was directly associated with the prevalence of frailty, slowness, and low physical activity (37). Wu et al. did not find any association between DAL and total mortality among never smokers but observed such association among past smokers (38).

Collectively, the above-mentioned factors may contribute to the relationship between high DAL and cancer incidence. However, all the studies reviewed have addressed this concern and considered the confounding effect of the factors in the DAL-cancer relationship analysis.

The exact mechanism connecting high DAL to the risk of cancer remains yet unclear. There are several potential hormonal and non-hormonal mechanistic pathways to demonstrate the long-term effect of diet-dependent acidosis on carcinogenesis as follows:

1) An acidosis diet May increase carcinogenesis by reduction of adiponectin secretion.

Adiponectin is a 244-amino acid protein secreted mainly by adipocytes and act as an endogenous insulin sensitizer. Low circulating adiponectin level is supposed to have a critical role in the development and progression of multiple malignancies (39). As shown in **Figure 4**, low adiponectin level contributes to increased insulin level, which in turn, leads to elevated levels of bioavailable insulin-like growth factor (IGF)-1 (40). Insulin and IGF-1 induce cellular proliferation and prevent apoptosis and are therefore involved in carcinogenesis (40).

A diet's protein content and origin may contribute to adiponectin production. Yagi et al. found that a low-protein diet significantly elevated serum adiponectin level and also increased the amount of adiponectin secreted by adipocytes isolated from white adipose tissue (41). Also, Ceolin et al. in an animal model study showed that serum adiponectin level was higher in animals fed with a low protein diet than standard protein diet (42). The results of a study on older women participating in a resistance-based exercise program showed that women with a high protein diet had significantly higher adiponectin content compared to those with a high carbohydrate diet (43). The source of protein may be a reason for the discrepancy observed in the studies finding. According to evidence, consumption of animal protein may reduce the level of adiponectin. Chen et al. reported that serum adiponectin level was lower in rats fed animal-based protein diet than rats fed vegan protein-based diets (44). Moreover, a large body of evidence indicates a positive relationship between high adherence to plant-based diets such as the Mediterranean diet and serum adiponectin level (45, 46). Furthermore, endogenous metabolic acidosis, as an outcome of DAL, may also lead to a reduced level of adiponectin. Disthabanchong et al. in *in-vivo* and *in-vitro* studies showed that metabolic acidosis prevented adiponectin gene expression and reduced adiponectin serum levels (47). There was no study to show the effect/association of DAL on/with adiponectin level.

Taken all together, consumption of animal-based protein and metabolic acidosis state in the body may diminish the level of adiponectin, which in turn, increases the risk of cancer. Further research is required to evaluate the effect/association of the acidosis diet on/with the adiponectin level.

2) An acidosis diet May increase carcinogenesis by elevation of cortisol production.

Cortisol is a stress hormone that controls numerous processes throughout the body, such as metabolism and performance of the immune system. A growing body of evidence is suggesting a positive relationship between high cortisol levels and the progression of cancer (48–50). High cortisol concentrations adversely suppress the immune system and decrease its sufficiency in eliminating mutated cells (48). In addition, higher cortisol concentrations may contribute to the development of cancer by increasing DNA damage and apoptosis suppression (48) (**Figure 4**).

The amount of protein in a diet or an acidifying diet or metabolic acidosis condition in the body may enhance the production of cortisol. Slag et al. showed that consumption of a high protein diet contributed to the increased release of cortisol in healthy individuals (51). Also, Lemmens et al. reported that consumption of a high-protein meal increased cortisol levels in men and women (52). Esche et al. suggested that the presence of a moderate increase in diet-dependent acid load is adequate to increase glucocorticoids secretion and influence cortisol metabolism (53). Buehlmeier et al. showed that diet-dependent acidification/alkalization influenced glucocorticoids activity and metabolism, in healthy men (54). Perez et al. in a study on dogs reported that metabolic acidosis was associated with increased plasma cortisol levels of animals (55).

3) An acidosis diet May increase carcinogenesis by elevation of circulating IGF-1 level.

The insulin-like growth factor is a hormone with a critical role in the growth and mediates the anabolic effects of growth hormone or protein synthesis in muscle and skeletal tissues. Elevated circulating IGF-1 level promotes tumorigenesis, angiogenesis, and metastasis (56–58). IGF-1 stimulates several signaling pathways such as PI3K/Akt and MAPK through binding to its cell surface receptor and induces cancer cell proliferation, survival, and migration (57) (**Figure 4**).

Diet protein level or acidity or metabolic acidosis state of the body may elevate the production of IGF-1. An extensive body of studies have consistently indicated that intake of a high protein diet up-regulates the IGF-1 level. Schüler et al. reported that consumption of a high protein diet significantly increased IGF-1 levels in patients with type 2 diabetes (59). Giovannucci et al., in a study on 753 men, reported that men with high total protein intakes had a 25% greater plasma IGF-1 level than those with low protein intake (60). Drake et al. found that high protein intake was associated with high plasma IGF-1 level, in women older than 50 years (61). Morgan et al. showed that low protein intake was associated with a reduced level of IGF-1 in the population aged $\leq$ 65 years (62). Wan et al. showed that serum IGF-1 and liver IGF-1 mRNA levels were lower in pigs fed with low-protein than pigs fed with normal crude protein (63). Regarding the source of protein, Hoppe et al. reported that serum IGF-1 level was significantly associated with intakes of animal protein and milk, but not with the intake of vegetable protein or meat (64). Schüler et al. reported that both animal and plant protein intake lead to significant increases of IGF-1 level, which was unchanged by the various amino acids plant and animal protein composition, in participants with type 2 diabetes (59).

Concerning the association between DAL and IGF-1 concentration, research is too scarce. In a study, Lim et al. did not find any interaction effects between DAL and IGF-1 (65). Moreover, several relatively archaic studies have indicated that NH4Cl-induced metabolic acidosis reduces IGF-1 (66, 67). Additional researches to examine the association between DAL and dietary-induced metabolic acidosis with serum levels of IGF-1 are needed to better understand how high protein intake may affect IGF-1 level.

4) An acidosis diet May increase carcinogenesis by elevation of insulin resistance.

Insulin resistance (IR) is a pathological condition that presents when a disturbance occurs in the biological response to insulin.

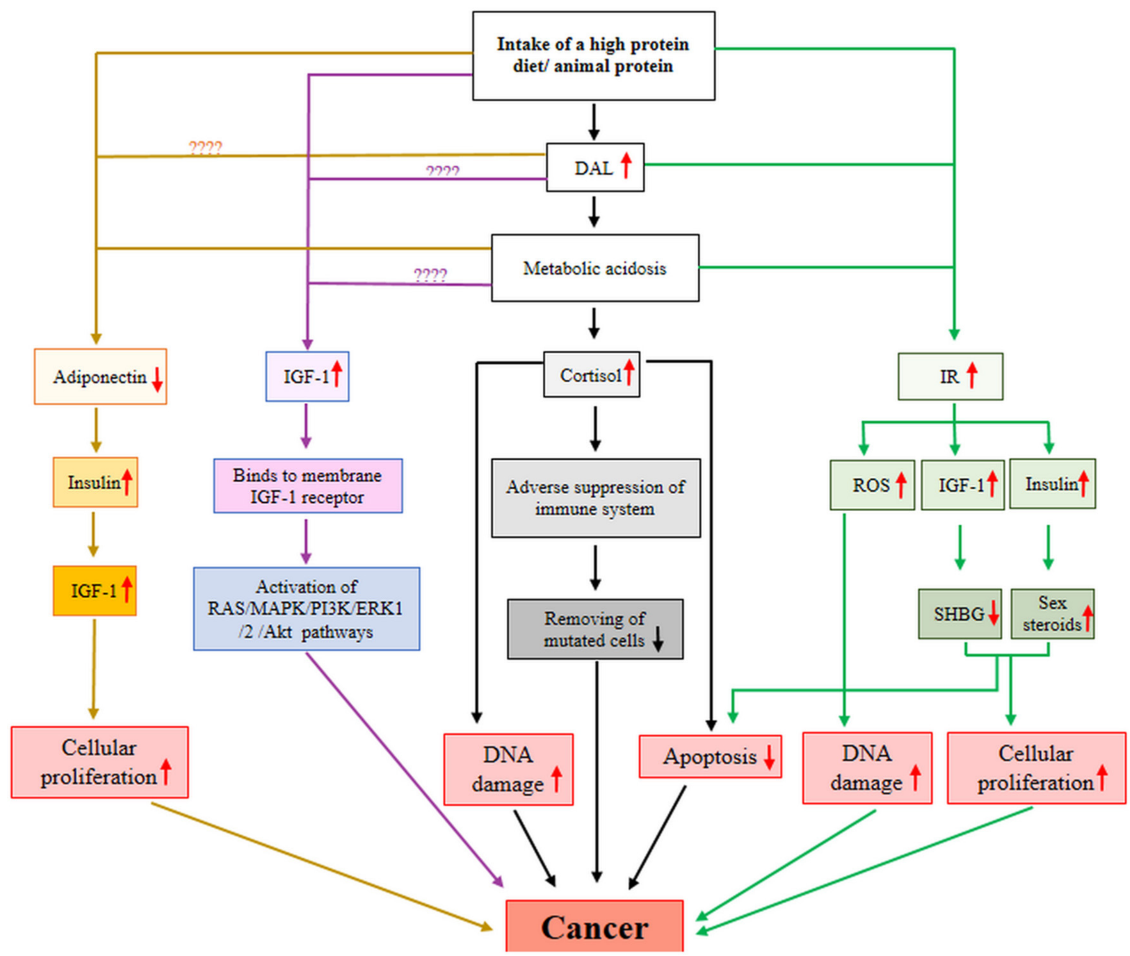

FIGURE 4 | A possible mechanistic model for DAL-cancer relationship. AKt, protein kinase B; DAL, dietary acid load; ERK1, extracellular signal-regulated kinase 1; IGF-1, insulin-like growth factor-1; IR, insulin resistance; MAPK, mitogen-activated protein kinase; PI3K, Phosphoinositide 3-kinases; ROS, reactive oxygen species; SHBG, sex-hormone binding globulin.

IR is well known to raise the risk of metabolic diseases such as cancer (68, 69). The possible mechanism for this association has fully been explained by Arcidiacono et al. (68). In brief, as shown in **Figure 4**, IR leads to hyperinsulinemia and enhancement of bioavailable IGF-1, which both of them prevent the hepatic production of sex-hormone binding globulin and induce ovarian production of sex steroids. Finally, these alterations stimulate cellular proliferation and prevent apoptosis (68). IR may contribute to carcinogenesis through impaired DNA due to excess production of reactive oxygen species (68).

Consumption of a high protein diet, DAL, and metabolic acidosis may impact IR level. Results of a systematic review and meta-analysis of randomized controlled trials showed that intake of a high-protein diet may reduce IR levels in patients with type 2 diabetes (70). Morenga et al., in an interventional study on overweight or obese women, found that insulin sensitivity reduced by 19.3% after intake of a diet relatively high in both protein and fiber compared with a standard diet (71). The source of protein is an important factor for the modifying of IR. Azemati et al. in a cross-sectional study on 548 participants showed that intake of total protein, animal protein, and the ratio of animal-to-plant protein intake were positively linked to IR, but plant protein was not (72). Adeva-Andany et al. in a review study discussed the contribution of animal protein intake on increased IR, in various population groups (73). Furthermore, Wojcik et al. in an animal model study showed that a high-protein casein diet (animal protein) had a minimal benefit in reduction of IR compared with a high-protein soy diet, or high-protein combined diet with animal and plant proteins (74).

Concerning the association between DAL and IR level, Lee et al., in a study on 5,406 participants, concluded that DAL was positively correlated to the development of IR (75). Also, Akter et al. in a study on 1732 workers found that high DAL was positively associated with IR (76). Endogenous metabolic acidosis is another DAL-related factor that may influence IR level. Williams et al. in a cross-sectional study found that individuals with IR had a higher level of fasting plasma lactate, a marker of metabolic acidosis (77). Bellasi et al. in a study on 145 patients with chronic kidney disease showed that rectification of metabolic acidosis ameliorates IR (78).

STRENGTHS AND LIMITATIONS OF THE STUDY

The inclusion of several prospective cohort studies with large sample sizes, in the present review, enhances the power of the findings. All the studies, except one, used the standard FFQ method to assess food intake, and all the studies used two validated measurements of PRAL and NEAP for DAL assessment which make it possible to compare results among studies. Studying various types of cancer across the studies was a limitation of the present study, which may impact the comparability of the findings. All the included studies were observational and there was no intervention study.

CONCLUSIONS

The findings indicate that higher DAL may be associated with a higher risk of cancer incidence across cancer types, study populations, both genders, both DAL assessment indicators (PRAL and NEAP), and also among both high- and low-risk age groups for cancer.

APPLICATION OF THE FINDINGS

This finding highlights that high DAL, which reveals the metabolic and nutritional status of an individual, may have long-term effects on human health. As a primary prevention strategy against cancer, the elevation of knowledge and attitudes of people at the community level, toward harms of diets with high acid load through training and advertising may navigate people to healthier dietary habits. Moreover, at the clinical level, the providing of dietary recommendations regarding foods with low DAL may be of help to prevent the development and progression of cancer.

SUGGESTIONS FOR FUTURE RESEARCH

Further evidence from interventional investigations is required to affirm findings from observational studies. Further researches are needed to evaluate: effect/association of the high DAL on/with the serum adiponectin and IGF-1 level, the relationship between dietary-induced metabolic acidosis with IGF-1, and the effect/association of high DAL on/with cellular proliferation, apoptosis, and signaling pathways involved in these events.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SK and MA contributed to the concept, design, interpretation of the data, and preparation of the manuscript. MK and VM contributed to the articles searching process, data extraction and analysis. SK is responsible for design, writing, and final content of the manuscript. All authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.816797/full#supplementary-material

REFERENCES

1. Ma X, Yu H. Cancer issue: global burden of cancer. *Yale J Biol Med.* (2006) 79:85.
2. *Cancer* - WHO | World Health Organization. Available online at: https://www.who.int/news-room/fact-sheets/detail/cancer (accessed March 4, 2022)
3. Khan N, Afaq F, Mukhtar H. Lifestyle as risk factor for cancer: evidence from human studies. *Cancer Lett.* (2010) 293:133–43. doi: 10.1016/j.canlet.2009.12.013
4. Tayyem RF, Bawadi HA, Shehadah I, Agraib LM, AbuMweis SS, Al-Jaberi T, et al. Dietary patterns and colorectal cancer. *Clin Nutr.* (2017) 36:848–52. doi: 10.1016/j.clnu.2016.04.029
5. Schwingshackl L, Hoffmann G. Adherence to mediterranean diet and risk of cancer: an updated systematic review and meta-analysis of observational studies. *Cancer Med.* (2015) 4:1933–47. doi: 10.1002/cam4.539
6. Azeem S, Gillani SW, Siddiqui A, Jandrajupalli SB, Poh V, Sulaiman SAS. Diet and colorectal cancer risk in Asia-a systematic review. *Asian Pac J Cancer Prev.* (2015) 16:5389-96. doi: 10.7314/APJCP.2015.16.13.5389
7. Mentella MC, Scaldaferri F, Ricci C, Gasbarrini A, Miggiano GAD. Cancer and mediterranean diet: a review. *Nutrients.* (2019) 11:2059. doi: 10.3390/nu11092059
8. Aghamohammadi V, Salari-Moghaddam A, Benisi-Kohansal S, Taghavi M, Azadbakht L, Esmaillzadeh A. Adherence to the MIND diet and risk of breast cancer: a case-control study. *Clin Breast Cancer.* (2021) 21:e158–64. doi: 10.1016/j.clbc.2020.09.009
9. Hietavala E, Stout J, Hulmi J, Suominen H, Pitkänen H, Puurtinen R, et al. Effect of diet composition on acid–base balance in adolescents, young adults and elderly at rest and during exercise. *Eur J Clin Nutr.* (2015) 69:399–404. doi: 10.1038/ejcn.2014.245
10. Scialla JJ, Anderson CA. Dietary acid load: a novel nutritional target in chronic kidney disease? *Adv Chronic Kidney Dis.* (2013) 20:141–9. doi: 10.1053/j.ackd.2012.11.001
11. Osuna-Padilla I, Leal-Escobar G, Garza-García C, Rodríguez-Castellanos F. Dietary acid load: mechanisms and evidence of its health repercussions. *Nefrologia.* (2019) 39:339–454. doi: 10.1016/j.nefro.2018.10.005
12. Welch AA, Mulligan A, Bingham SA, Khaw K. Urine pH is an indicator of dietary acid–base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study. *Br J Nutr.* (2008) 99:1335–43. doi: 10.1017/S0007114507862350
13. Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am J Clin Nutr.* (1994) 59:1356–61. doi: 10.1093/ajcn/59.6.1356
14. Frassetto LA, Todd KM, Morris Jr RC, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr.* (1998) 68:576-83. doi: 10.1093/ajcn/68.3.576

15. Mozaffari H, Namazi N, Larijani B, Bellissimo N, Azadbakht L. Association of dietary acid load with cardiovascular risk factors and the prevalence of metabolic syndrome in Iranian women: a cross-sectional study. *Nutrition.* (2019) 67-68:110570. doi: 10.1016/j.nut.2019.110570
16. Parohan M, Sadeghi A, Nasiri M, Maleki V, Khodadost M, Pirouzi A, et al. Dietary acid load and risk of hypertension: a systematic review and dose-response meta-analysis of observational studies. *Nutr Metab Cardiovasc Dis.* (2019) 29:665–75. doi: 10.1016/j.numecd.2019.03.009
17. López M, Moreno G, Lugo G, Marcano G. Dietary acid load in children with chronic kidney disease. *Eur J Clin Nutr.* (2020) 74:57–62. doi: 10.1038/s41430-020-0687-3
18. Abshirini M, Bagheri F, Mahaki B, Siassi F, Koohdani F, Safabakhsh M, et al. The dietary acid load is higher in subjects with prediabetes who are at greater risk of diabetes: a case-control study. *Diabetol Metab Syndr.* (2019) 11:52. doi: 10.1186/s13098-019-0447-5
19. Jafari Nasab S, Rafiee P, Bahrami A, Rezaeimanesh N, Rashidkhani B, Sohrab G, et al. Diet-dependent acid load and the risk of colorectal cancer and adenoma: a case-control study. *Public Health Nutr.* (2020) 24:1-8. doi: 10.1017/S1368980020003420
20. Mehranfar S, Jalilpiran Y, Jafari A, Mohajeri SAR, Faghih S. *Dietary Acid Load and Risk of Prostate cancer: (A Case-Control Study).* Shiraz: Authorea (2020).
21. Park YM, Steck SE, Fung TT, Merchant AT, Elizabeth Hodgson M, Keller JA, et al. Higher diet-dependent acid load is associated with risk of breast cancer: findings from the sister study. *Int J Cancer.* (2019) 144:1834–43. doi: 10.1002/ijc.31889
22. Ronco A, Martínez-López W, Calderón J, Mendoza B. Dietary acid load and colorectal cancer risk: a case-control study. *World Cancer Res J.* (2020) 7:e1750. doi: 10.1016/S0959-8049(20)30866-2
23. Ronco AL, Martínez-López W, Calderón JM, Golomar W. Dietary acid load and lung cancer risk: a case-control study in men. *Cancer Treat Res Commun.* (2021) 28:100382. doi: 10.1016/j.ctarc.2021.100382
24. Shi LW, Wu YL, Hu JJ, Yang PF, Sun WP, Gao J, et al. Dietary acid load and the risk of pancreatic cancer: a prospective cohort study. *Cancer Epidemiol Biomarkers Prev.* (2021) 30:1009–19. doi: 10.1158/1055-9965.EPI-20-1293
25. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ.* (2021) 372:n160. doi: 10.1136/bmj.n160
26. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
27. DerSimonian R, Laird N. Meta-analysis in clinical trials revisited. *Contemp Clin Trials.* (2015) 45:139-45. doi: 10.1016/j.cct.2015.09.002
28. van Enst WA, Ochodo E, Scholten RJ, Hooft L, Leeflang MM. Investigation of publication bias in meta-analyses of diagnostic test accuracy: a meta-epidemiological study. *BMC Med Res Methodol.* (2014) 14:70. doi: 10.1186/1471-2288-14-70
29. Lin L. Hybrid test for publication bias in meta-analysis. *Stat Methods Med Res.* (2020) 29:2881–99. doi: 10.1177/0962280220910172
30. Wu T, Hsu FC, Pierce JP. Increased acid-producing diet and past smoking intensity are associated with worse prognoses among breast cancer survivors: a prospective cohort study. *J Clin Med.* (2020) 9:1817. doi: 10.3390/jcm9061817
31. Mousavi SM, Milajerdi A, Sshayanfar M, Esmaillzadeh A. Relationship between dietary acid load and Glioma: a case-control study. *Qom Univ Med Sci J.* (2019) 13:11–20. doi: 10.29252/qums.13.1.11
32. Ronco AL, Martínez-López W, Mendoza B, Calderón JM. Epidemiologic evidence for association between a high dietary acid load and the breast cancer risk. *SciMedicine J.* (2021) 3:166–76. doi: 10.28991/SciMedJ-2021-0302-8
33. Safabakhsh M, Imani H, Yaseri M, Omranipour R, Shab-Bidar S. Higher dietary acid load is not associated with risk of breast cancer in Iranian women. *Cancer Rep.* (2020) 3:e1212. doi: 10.1002/cnr2.1212
34. Hietavala EM, Stout JR, Frassetto LA, Puurtinen R, Pitkänen H, Selänne H, et al. Dietary acid load and renal function have varying effects on blood acid-base status and exercise performance across age and sex. *Appl Physiol Nutr Metab.* (2017) 42:1330–40. doi: 10.1139/apnm-2017-0279
35. Fatahi S, Qorbani M, Surkan PJ, Azadbakht L. Associations between dietary acid load and obesity among Iranian women. *J Cardiovasc Thorac Res.* (2021) 13:285–97. doi: 10.34172/jcvtr.2021.44
36. Li Y, He Y, Wang D, Gao X. Association between dietary acid-based load and obesity in Chinese adults. *Exp Biol.* (2012) 26:826.4. doi: 10.1096/fasebj.26.1_supplement.826.4
37. Kataya Y, Murakami K, Kobayashi S, Suga H, Sasaki S, Three-generation Study of Women on Diets and Health Study Group. Higher dietary acid load is associated with a higher prevalence of frailty, particularly slowness/weakness and low physical activity, in elderly Japanese women. *Eur J Nutr.* (2018) 57:1639–50. doi: 10.1007/s00394-017-1449-4
38. Wu T, Hsu F, Wang SS, John Pierece J. Dietary acid load, smoking intensity and total mortality. *Curr Dev Nutr.* (2020) 4:89. doi: 10.1093/cdn/nzaa040_089
39. Dalamaga M, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocr Rev.* (2012) 33:547–94. doi: 10.1210/er.2011-1015
40. Kelesidis I, Kelesidis T, Mantzoros CS. Adiponectin and cancer: a systematic review. *Br J Cancer.* (2006) 94:1221–5. doi: 10.1038/sj.bjc.6603051
41. Yagi T, Toyoshima Y, Tokita R, Taguchi Y, Okamoto Y, Takahashi S, et al. Low-protein diet enhances adiponectin secretion in rats. *Biosci Biotechnol Biochem.* (2019) 83:1774–81. doi: 10.1080/09168451.2019.1621153
42. Ceolin P, DE França SA, Froelich M, Santos MPD, Pereira MP, Queiroz TS, et al. A low-protein, high carbohydrate diet induces increase in serum adiponectin and preserves glucose homeostasis in rats. *An Acad Bras Cienc.* (2019) 91:e20180452. doi: 10.1590/0001-3765201920180452
43. Galbreath M, Campbell B, Bounty PL, Bunn J, Dove J, Harvey T, et al. Effects of adherence to a higher protein diet on weight loss, markers of health, and functional capacity in older women participating in a resistance-based exercise program. *Nutrients.* (2018) 10:1070. doi: 10.3390/nu10081070
44. Chen JH, Song J, Chen Y, Ding Q, Peng A, Mao L. The effect of vegan protein-based diets on metabolic parameters, expressions of adiponectin and its receptors in wistar rats. *Nutrients.* (2016) 8:643. doi: 10.3390/nu8100643
45. Izadi V, Azadbakht L. Specific dietary patterns and concentrations of adiponectin. *J Res Med Sci.* (2015) 20:178–84.
46. Sureda A, Bibiloni MM, Julibert A, Bouzas C, Argelich E, Llompart I, et al. Adherence to the Mediterranean diet and inflammatory markers. *Nutrients.* (2018) 10:62. doi: 10.3390/nu10010062
47. Disthabanchong S, Niticharoenpong K, Radinahamed P, Stitchantrakul W, Ongphiphadhanakul B, Hongeng S. Metabolic acidosis lowers circulating adiponectin through inhibition of adiponectin gene transcription. *Nephrol Dial Transplant.* (2011) 26:592–8. doi: 10.1093/ndt/gfq410
48. Al Sorkhy M, Fahl Z, Ritchie J. Cortisol and breast cancer: a review of clinical and molecular evidence. *Ann Cancer Res Ther.* (2018) 26:19–25. doi: 10.4993/acrt.26.19
49. Fabre B, Grosman H, Gonzalez D, Machulsky NF, Repetto EM, Mesch V, et al. Prostate cancer, high cortisol levels and complex hormonal interaction. *Asian Pac J Cancer Prev.* (2016) 17:3167–71. doi: 10.14456/apjcp.2016.70
50. Bernabé DG, Tamae AC, Miyahara GI, Sundefeld MLM, Oliveira SP, Biasoli ER. Increased plasma and salivary cortisol levels in patients with oral cancer and their association with clinical stage. *J Clin Pathol.* (2012) 65:934–9. doi: 10.1136/jclinpath-2012-200695
51. Slag MF, Ahmad M, Gannon MC, Nuttall FQ. Meal stimulation of cortisol secretion: a protein induced effect. *Metabolism.* (1981) 30:1104–8. doi: 10.1016/0026-0495(81)90055-X
52. Lemmens SG, Born JM, Martens EA, Martens MJ, Westerterp-Plantenga MS. Influence of consumption of a high-protein vs. high-carbohydrate meal on the physiological cortisol and psychological mood response in men and women. *PLoS ONE.* (2011) 6:e16826. doi: 10.1371/journal.pone.0016826
53. Esche J, Shi L, Sánchez-Guijo A, Hartmann MF, Wudy SA, Remer T. Higher diet-dependent renal acid load associates with higher glucocorticoid secretion and potentially bioactive free glucocorticoids in healthy children. *Kidney Int.* (2016) 90:325–33. doi: 10.1016/j.kint.2016.02.033
54. Buehlmeier J, Remer T, Frings-Meuthen P, Maser-Gluth C, Heer M. Glucocorticoid activity and metabolism with NaCl-induced low-grade metabolic acidosis and oral alkalization: results of two randomized controlled trials. *Endocrine.* (2016) 52:139–47. doi: 10.1007/s12020-015-0730-7

55. Perez GO, Oster JR, Katz FH, Vaamonde CA. The effect of acute metabolic acidosis on plasma cortisol, renin activity and aldosterone. *Horm Res.* (1979) 11:12–21. doi: 10.1159/000179033
56. Watts EL, Fensom GK, Byrne KS, Perez-Cornago A, Allen NE, Knuppel A, et al. Circulating insulin-like growth factor-I, total and free testosterone concentrations and prostate cancer risk in 200 000 men in UK Biobank. *Int J Cancer.* (2021) 148:2274–88. doi: 10.1002/ijc.33416
57. Hua H, Kong Q, Yin J, Zhang J, Jiang Y. Insulin-like growth factor receptor signaling in tumorigenesis and drug resistance: a challenge for cancer therapy. *Hematol Oncol.* (2020) 13:64. doi: 10.1186/s13045-020-00904-3
58. Murphy N, Knuppel A, Papadimitriou N, Martin RM, Tsilidis KK, Smith-Byrne K, et al. Insulin-like growth factor-1, insulin-like growth factor-binding protein-3, and breast cancer risk: observational and Mendelian randomization analyses with ∼430 000 women. *Ann Oncol.* (2020) 31:641–9. doi: 10.1016/j.annonc.2020.01.066
59. Schüler R, Markova M, Osterhoff MA, Arafat A, Pivovarova O, Machann J, et al. Similar dietary regulation of IGF-1- and IGF-binding proteins by animal and plant protein in subjects with type 2 diabetes. *Eur J Nutr.* (2021) 60:3499–504. doi: 10.1007/s00394-021-02518-y
60. Giovannucci E, Pollak M, Liu Y, Platz EA, Majeed N, Rimm EB, et al. Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. *Cancer Epidemiol Biomarkers Prev.* (2003) 12:84–9.
61. Drake KN, Foganholo J, Brindisi A, Samavat H, Sturgeon K, Schmitz K, et al. Protein intake is associated with plasma insulin-like growth factor (IGF)1 in postmenopausal women but not in premenopausal women. *FASEB J.* (2016) 30:1164.3. doi: 10.1096/fasebj.30.1_supplement.1164.3
62. Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, et al. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab.* (2014) 19:407–17. doi: 10.1016/j.cmet.2014.02.006
63. Wan X, Wang S, Xu J, Zhuang L, Xing K, Zhang M, et al. Dietary protein-induced hepatic IGF-1 secretion mediated by PPARγ activation. *PLoS ONE.* (2017) 12:e0173174. doi: 10.1371/journal.pone.0173174
64. Hoppe C, Udam TR, Lauritzen L, Mølgaard C, Juul A, Michaelsen KF. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. *Am J Clin Nutr.* (2004) 80:447–52. doi: 10.1093/ajcn/80.2.447
65. Lim SY, Chan YM, Ramachandran V, Shariff ZM, Chin YS, Arumugam M. Dietary acid load and its interaction with IGF1 (rs35767 and rs7136446) and IL6 (rs1800796) polymorphisms on metabolic traits among postmenopausal women. *Nutrients.* (2021) 13:2161. doi: 10.3390/nu13072161
66. Brüngger M, Hulter HN, Krapf R. Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis: new cause of growth hormone insensitivity in humans. *Kidney Int.* (1997) 51:216–21. doi: 10.1038/ki.1997.26
67. Green J, Maor G. Effect of metabolic acidosis on the growth hormone/IGF-I endocrine axis in skeletal growth centers. *Kidney Int.* (2000) 57:2258–67. doi: 10.1046/j.1523-1755.2000.00086.x
68. Arcidiacono B, Iiritano S, Nocera A, Possidente K, Nevolo MT, Ventura V, et al. Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. *Exp Diabetes Res.* (2012) 2012:789174. doi: 10.1155/2012/789174
69. Alan O, Telli TA, Aktas B, Koca S, Ökten IN, Hasanov R, et al. Is insulin resistance a predictor for complete response in breast cancer patients who underwent neoadjuvant treatment? *World J Surg Oncol.* (2020) 18:242. doi: 10.1186/s12957-020-02019-y
70. Yu Z, Nan F, Wang LY, Jiang H, Chen W, Jiang Y. Effects of high-protein diet on glycemic control, insulin resistance and blood pressure in type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Clin Nutr.* (2020) 39:1724–34. doi: 10.1016/j.clnu.2019.08.008
71. Morenga LT, Docherty P, Williams S, Mann J. The effect of a diet moderately high in protein and fiber on insulin sensitivity measured using the dynamic insulin sensitivity and secretion test (DISST). *Nutrients.* (2017) 9:1291. doi: 10.3390/nu9121291
72. Azemati B, Rajaram S, Jaceldo-Siegl K, Sabate J, Shavlik D, Fraser GE, et al. Animal-protein intake is associated with insulin resistance in Adventist Health Study 2 (AHS-2) calibration substudy participants: a cross-sectional analysis. *Curr Dev Nutr.* (2017) 1:e000299. doi: 10.3945/cdn.116.000299
73. Adeva-Andany MM, González-Lucán M, Fernández-Fernández C, Carneiro-Freire N, Seco-Filgueira M, Pedre-Piñeiro AM. Effect of diet composition on insulin sensitivity in humans. *Clin Nutr ESPEN.* (2019) 33:29–38. doi: 10.1016/j.clnesp.2019.05.014
74. Wojcik JL, Devassy JG, Wu Y, Zahradka P, Taylor CG, Aukema HM. Protein source in a high-protein diet modulates reductions in insulin resistance and hepatic steatosis in fa/fa Zucker rats. *Obesity.* (2016) 24:123–31. doi: 10.1002/oby.21312
75. Lee KW, Shin D. Positive association between dietary acid load and future insulin resistance risk: findings from the Korean Genome and Epidemiology Study. *Nutr J.* (2020) 19:137. doi: 10.1186/s12937-020-00653-6
76. Akter S, Eguchi M, Kuwahara K, Kochi T, Ito R, Kurotani K, et al. High dietary acid load is associated with insulin resistance: the Furukawa Nutrition and Health Study. *Clin Nutr.* (2016) 35:453–9. doi: 10.1016/j.clnu.2015.03.008
77. Williams RS, Heilbronn LK, Chen DL, Coster ACF, Greenfield JR, Samocha-Bonet D. Dietary acid load, metabolic acidosis and insulin resistance - lessons from cross-sectional and overfeeding studies in humans. *Clin Nutr.* (2016) 35:1084–90. doi: 10.1016/j.clnu.2015.08.002
78. Bellasi A, Micco LD, Santoro D, Marzocco S, Simone ED, Cozzolino M, et al. Correction of metabolic acidosis improves insulin resistance in chronic kidney disease. *BMC Nephrol.* (2016) 17:158. doi: 10.1186/s12882-016-0372-x

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Nutrient and Nitrate Composition of Greenhouse-Grown Leafy Greens: A Trial Comparison Between Conventional and Organic Fertility Treatments

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Arugula (*Eruca sativa*), mizuna (*Brassica rapa* var. *nipponsinca*), red giant mustard (*Brassica juncea*), and spinach (*Spinaciaoleracea* "Tyee") are fresh produce crops high in nutritive value that provide shortfall and high interest nutrients addressed in the U.S. Dietary Guidelines. The primary objective of this project was to evaluate fertility treatments unique to these crops that optimize their nutritional capacity. Measurements discussed include: vitamin C, dietary fiber, calcium, iron, potassium, sodium, and nitrate. Plants were grown at the University of Minnesota St. Paul Campus (St. Paul, MN) in a greenhouse from November to April under an 18 h photoperiod and a 24/13°C day/night temperature. Plants were grown using five different fertility treatments, including four organic treatments and one conventional control. The plant treatment combinations were replicated three times and the entire experiment was duplicated. Fertility treatments had a high impact on vitamin C (with over a 3-fold difference in treatments in the first experiment), nitrate (over 10-fold difference among fertility treatments in some species) and potassium concentrations (over 5-fold difference among fertility treatments in some species) in analyzed plant tissue. No consistent differences were found for fiber, calcium, iron and sodium concentrations in tissue analyzed. This is the first study to analyze the impact that different organic treatments can have on multiple nutrients and compounds addressed by the U.S. Dietary Guidelines for high-impact, highly-consumed produce crops.

Keywords: organic, nitrate, vitamin C, dietary fiber, minerals, leafy greens

INTRODUCTION

Diets high in fruits and vegetables are widely recommended for their health promoting properties (Slavin and Lloyd, 2012). Fruits and vegetables contain vitamin, minerals, electrolytes, dietary fiber, and phytochemicals especially antioxidants. Although the positive aspects of consumption of fruits and vegetables are promoted, vegetables especially leafy green vegetables are concentrated in nitrates that have been linked to adverse health effects especially in children (Karnpanit et al., 2018). Production practices that increase the nutrient content of vegetables while limiting high amounts of potential negative compounds such as nitrates are of interest.

Organic fruits and vegetables have dominated the organic market in the past three plus decades (Donaldson, 2021). In 2014, organic food sales measured more than twice those of 1994. A 2016 survey found that 68% of Americans had purchased organic food in the previous month. Seventy-one percent of Americans reported that nutrition and ingredients labels were factors in choosing which foods to buy. Additionally, 63% of respondents who paid particular attention to consuming healthy and nutritious foods reported that organic food composes at least some of their intake. On the other hand, in the respondents who did not pay particular attention to consuming healthy and nutritious foods, 22% reported that organic food composes at least some of their food intake (Funk and Kennedy, 2016).

Consumers mainly buy organic due to the alignment of their beliefs with the ideology behind organic production. Accordingly, the above mentioned 2016 survey determined that individuals very concerned about genetically modified foods tended to have chosen organic and GMO-free labeled foods in the previous month. Furthermore, the same 2016 survey reported that 76% of those who had purchased organic in the previous month cited health as motivation. Comparatively, 33% of this same subset claimed environmental reasons, and 22% reported convenience. Finally, 72% of Americans make organic vs. conventional purchase decisions by considering price differences (Funk and Kennedy, 2016).

Almost 75% of conventional grocery stores and close to 20,000 natural food stores sell organic goods (Donaldson, 2021), Generally organic foods are more expensive and more likely to be purchased by higher income consumers (Mie et al., 2017). Lack of access to organic foods for certain populations opposes one of the four principles of organic agriculture: fairness. This principle maintains that organic agriculture should foster equity and justice (IFOAM-Organics International, 2020).

Overall, organic farming principles and practices aim to create a food system that utilizes the biological cycles and resources of the earth in a way that protects biodiversity and preserves the balance of the ecosystem (Hunter et al., 2011). Key fundamentals of traditional organic production encourage long-term fertility of soils, minimize carbon footprints and maintain genetic diversity in current food systems.

In enclosed environment greenhouse systems, key organic practices are implemented, like the use of non-synthetic fertilizers and pesticides, although they are often not complete replacements of traditional organic practices used in open field environments. For example, in open-field organic farming, concern for health of the soil necessitates use of cover crops. Yet in greenhouse production, cover crops are not generally employed.

Differences in organically and conventionally produced foods have been extensively reviewed, based on their nutritive value, sensory qualities and overall safety, but limited information exists on differences in crops grown in greenhouses. Arugula (*Eruca sativa*), mizuna (*Brassica rapa*var. *nipposinica*), and red giant mustard (*Brassica juncea*) are Mesclun mixture plants that are nutrient-dense and consumed in many regions. Spinach (*Spinacia oleracea*) is commonly consumed as both a plate vegetable and salad green (Lucier et al., 2004). Consumption of fresh lettuces and spinach, termed microgreens has increased greatly, but little information exists on the nutrient content of these greens, especially when grown in greenhouses (Mir et al., 2017).

Minerals play many critical roles in human physiology and are responsible for a wide range of activities in the body. In plants, iron plays a critical role as a cofactor in chloroplast biosynthesis (Soetan et al., 2010). Calcium is known for its ability to aid in the formation of stable cell walls and membranes, and regulates stimulus of cells (Soetan et al., 2010). Potassium primarily acts as a cofactor for protein synthesis and is a major solute in maintaining water balance and osmosis (Soetan et al., 2010). In the human body, iron, calcium, and potassium are essential nutrients often under consumed.

In plants, vitamin C plays many roles, and the content of Vitamin C in plants varies greatly depending on cultivar, growing conditions, maturity at harvest, and postharvest handling, processing, and storage (Phillips et al., 2018). In the human body, vitamin C plays many roles, including the ability to act as a water-soluble antioxidant, aid in collagen synthesis, increase absorption of iron from the diet and plays other critical roles in the metabolism of folate and some amino acids (Phillips et al., 2018). Dietary fiber is a critical shortfall nutrient in the United States. The typical U.S. individual consumes 17 g/d while the Daily Value is 28 g/day (Slavin, 2013).

Nitrate is a chemical substance naturally found in plants and heavy use of chemical fertilizers, especially nitrogen to increase crop yield increases nitrate levels in plants (Karnpanit et al., 2018). Many vegetables, especially green leafy vegetables contain high amounts of nitrate (Leon and Luzardo, 2020). Nitrate is generally considered safe to consume in moderate amounts and is easily converted to nitrite through reduction. Although nitrate and nitrite are not carcinogenic themselves, they can easily yield carcinogenic compounds. Nitrate reacts with secondary and tertiary amines endogenously, forming N-nitroso compounds (Walker, 2009; Karwowska and Kononiuk, 2020). N-nitroso compounds have been associated with higher risk of developing esophagus, stomach and liver carcinomas. Nitrate levels of vegetables are particularly of concern in childrens' diets (Karnpanit et al., 2018).

Nitrate accumulation is common in Brassica plants as well as other leafy vegetables such as spinach and lettuce and is influenced heavily by nitrogen fertilization practices (Hamdard et al., 2009). Cultivation practices are known to affect nitrate levels in vegetables (Karnpanit et al., 2018). Nitrate contents of most leafy vegetable grown under organic and and GAP (good agricultural practice) cultivation in Thailand were lower than those grown with conventional production (Karnpanit et al., 2018). Other surveys of nitrate concentrations in conventional and organic-labels raw vegetables at retail find that samples of fresh broccoli, cabbage, celery, lettuce and spinach categorized as conventional or organic by label in 5 major cities in

TABLE 1 | Fertility treatment combinations of media, fertility sources, and bulk densities of compared media.

Treatment	Media base	Fertilizer	Bulk density (g/cm^3)
All-in-one potting mix (AO)	Purple Cow Organic Potting Mix (Purple Cow Organics, Middleton, WI)	None	0.307
Custom mix (CM)	Peat, vermiculite, leaf litter compost (3:2:3 mix by volume)	Greensand, rock phosphate, bloodmeal and lime (1:1:1:0.5 mix by volume).	0.181
Conventional comparison (CC)	SunGro LC8 (SunGro Horticulture, Agawam, MA)	Peter's Excel CalMag 15-5-15 (Everris, Dublin, OH)	0.138
Fish emulsion (FE)	Sunshine Natural and Organic Planting Mix (SunGro Horticulture, Agawam, MA)	Dramatic 2-5-0.2 (Dramm, Manitowoc, WI)	0.086
Poultry litter (PL)	Sunshine Natural and Organic Planting Mix (SunGro Horticulture, Agawam, MA)	SUSTANE 8-4-4 (Sustane, Cannon Falls, MN)	0.086

diffierent geographic regions of the United States showed no differences in mean nitrate values (Nunez de Gonzalez et al., 2015). Yet in most cases, organic vegetables were numerically lower in nitrate content than their conventional counterparts in their analysis. A study of nitrate contents in regulated and non-regulated leafy vegetables of high consumption in the Canary islands, Spain reported that levels of nitrates in organic vegetables were significantly higher than those of conventional cultivation for chard and watercress (Leon and Luzardo, 2020). No seasonal differences were observed and overall nitrate levels were lower than those reported in other studies. Nitrate levels in organically grown crops were reviewed by Baranski et al. (2014) who concluded that nitrate levels are generally higher in conventionally grown crops because of high mineral nitrogen inputs.

The purpose of this project was to address differences in nutrients of interest in greenhouse-grown arugula, red giant mustard, mizuna and spinach. Specifically we are interested in how nutrient composition of these species is affected by various organic amendments along with a conventional comparison. These four plants were chosen because they have a high nutrient density and they have high amounts of common shortfall nutrients. In addition, they are of interest to growers in cold climates like Minnesota for winter greenhouse production and they are frequently consumed by many populations worldwide. We also determined nitrate content of these leafy green vegetables, both grown conventionally and organically.

MATERIALS AND METHODS

Plants

Arugula (*Eruca sativa*), mizuna (*Brassica rapa* var. *nipponsinca*), red giant mustard (*Brassica juncea*), and spinach (*Spinacia oleracea* "Tyee") were grown on five different fertilizer/media combinations. All seed for this study was obtained from Johnny's Selected Seeds (Winslow, ME). Arugula, mizuna, and red giant mustard seeds were sown into standard 1,020 trays (28 × 54 × 6 cm) at a density of 11 mL per tray. Spinach was sown into 50 cell deep plug trays with two seeds per cell, plants were thinned to one plant per cell after germination. All four species were grown on all five fertilizer/media combinations and replicated three times. The entire experiment was conducted twice, once starting in November 2014 and once starting in February 2015. Plants were watered by hand as needed. The experiment was set up using a randomized complete block design.

Fertility Treatments

Media and fertility treatments (**Table 1**) were mixed prior to planting. Fertility was scaled to meet field nitrogen requirements for crops on low organic matter soils according to University of Minnesota Extension materials (Rosen and Eliason, 2005). Treatments were fertilized by converting the recommended kg N/ha to kg N/m^3 using a depth of 15 cm, then calculating the weight of solid fertilizer or volume of liquid fertilizer to apply to the volume of media in each container using the guaranteed analysis of each fertilizer product. Both the custom mix and all-in-one potting mix exceeded nitrogen recommendations and so these treatments were not further amended. Full nitrogen recommendation was the equivalent of 112 kg/ha for leafy greens. Bulk densities of the compared media ranged from 0.086 to 0.307 g/cm^3 for the treatment groups (**Table 1**). Chemical analysis of media nutrients was conducted for complete comparison of soil nutrients (**Table 2**). Greens were grown to baby leaf lettuce size, and so they did not require the second application.

Plant Harvest

All plants were grown on benches in a greenhouse maintained with a 24°C day temperature and 13°C night temperature. Benches were blocked by treatment. Greens were harvested when the majority of leaves were 10 cm long by cutting with scissors. Greens were harvested between 1 and 3 times depending on plant vigor. All plant tissue and soil data were taken at time of first harvest. Harvest occurred between 4 and 9 weeks after planting. Tissue samples for vitamin C and dietary fiber analysis were frozen immediately at −80°C. Tissue samples for mineral analysis were dried to completion at 60°C, and crosschecked to control for moisture removal completion.

Comparison plants were grown at three greenhouses in Minnesota that participated as volunteers in this study. Growers were provided with packets of detailed instructions for the experiment and provided with all required materials. Regular

TABLE 2 | Soil nutrient profile comparisons for five fertility treatments analyzed.

	CC	AO	CM	FE	PL
	Mineral data below as mg/kg dry soil				
pH	5.90 (0.20)	6.75 (0.25)	6.40 (0.08)*	6.15 (0.05)	6.25 (0.05)
Soluble salts (dS/m)	3.08 (0.50)	3.57 (1.05)	10.20 (1.18)*	1.78 (0.27)	1.39 (0.27)
NO_3-N	3,734 (714)	3,617 (2,410)	1,492 (1,418)*	1,010 (5.44)	Below detection limit
NH4-N	225 (32)	13.74 (5.16)	352 (106)*	433 (26)	223 (71)
P	141 (13)	346 (27)	110 (15)*	614 (39)	140 (31)
K	1,099 (157)	2,970 (123)	10,330 (684)*	421 (62)	457 (131)
Fe	3.23 (0.23)	1.59 (0.20)	3.62 (0.94)*	2.15 (0)**	1.23 (0)**
Ca	2,051 (167)	1,462 (117)	2,333 (221)*	1,155 (78)	928 (102)
Mg	1,675 (69)	754 (120)	715 (62)*	1,180 (175)	906 (124)
B	1.01 (0.15)	2.89 (0.25)	4.43 (0.60)*	Below detection limit	Below detection limit
Na	182 (10)	635 (24)	215 (16)*	674 (100)	279 (40)
Mn	5.60 (2.6)	2.70 (1.1)	7.38 (1.2)*	2.89 (0.26)	2.43 (0.26)
Mo	0.21 (0)**	0.22 (0.04)	0.22 (0.08)	Below detection limit	0.25 (0)**
Zn	1.90 (0.24)	0.5 (0.07)	0.67 (0.05)*	0.72 (0)	0.59 (0.15)

*Data shown are mean (SD). Data shown are n = 2 unless otherwise noted (*n = 3, **n = 1).*
CC, Conventional Control; AO, All-in-One Potting Mix; CM, Custom Mix; FE, Fish Emulsion; PL, Poultry Litter.

visits were scheduled with research staff to ensure compliance and conduct analysis.

Media and Tissue Analysis

Exchangeable calcium was extracted from the media by mixing 10 mL or 1 molar, pH 7, ammonium acetate with 10 g of air-dried sample, and then placed in a shaker for 5 min. The filtered extract was analyzed with an inductively coupled plasma atomic emission spectrometer (ICP-AES). Extractable iron was determined by treating a 10 g sample of air-dried media with 20 mL of DTPA (Diethylenetriamine-pentaacetic acid) extracting solution. Samples were placed in a shaker for 2 h, and then filtered and analyzed with an ICP-AES for iron concentration of media. Available potassium was extracted from the media by mixing 10 mL of 1 molar ammonium acetate, pH7, with 1 g of air-dried media and then placed in a shaker for 5 min. Available potassium was then measured by analyzing the filtered extract on an ICP-AES set on emission mode at 776 nm. Nitrate-nitrogen was determined by adding 60 mL of KCl extracting solution to a 2 g sample of air-dried media, and then placed in a shaker for 15 min. The nitrate level in the filtered extract was measured on a Lachat QuckChem 8,500 Flow Injection Analyzer by the cadmium reduction method. Bulk densities of media were calculated according to Grafton et al. with minor revisions (Grafton et al., 2015). Plant tissue minerals (calcium, iron, potassium and sodium) were analyzed by weighing 500 mg of air-dried tissue into a 20 mL high form silica crucible and dry ashed at 485°C for 12 h (covered). Ash was then mixed with 5 mL of 20% HCl at room temperature for 30 min, followed by an addition of 5 mL of deionized water, then allowed to settle for 3 h prior to ICP-AES analysis. Plant tissue nitrate-nitrogen was extracted by shaking 300 mg of dried sample with 30 mL 0.1 m $CaSO_4$ solution for 30 min, followed by the addition of 0.85 cc of prewashed charcoal, followed by shaking for an additional 5 min. Samples were filtered through Whatman (No. 42) filter paper and nitrate concentrations in the filtrate were determined colorimetrically by the cadmium reduction method.

Vitamin C and Fiber Quantitation

Vitamin C was quantified using the AOAC 967.22 method from 50 g samples of frozen (−80°C) plant tissue. Total dietary fiber was quantified using the AOAC 991.43 method from 100 g samples of frozen (−80°C) plant tissue.

Statistical Analysis

All statistical analysis was conducted using SPSS (SPSS Chicago, IL). Analysis of variance (ANOVA) with Tukey HSD was used for all tests measuring differences among means. Log transformations were applied where necessary based on regression fit. Statistical significance was achieved for p-values < 0.05.

Comparison of Plants Grown in Experiment 1 and Experiment 2

ANOVA indicated that there were significant differences between experiment 1 and experiment 2 for fertility treatment interactions ($p < 0.001$), so biomass, nitrate and nutrient levels were analyzed separately for each experiment.

RESULTS

Harvested Biomass

Harvested biomass for arugula varied among the five treatments in experiment 1 (**Figure 1**). Custom Mix (CM) had the highest harvested biomass (598.5 g/m^2), which was significantly more than the Fish Emulsion (FE) treatment (330.7 g/m^2; $p = 0.039$). The CM, All-in-One Potting Mix (AO), Conventional Comparison (CC), and Poultry Litter (PL) treatments were

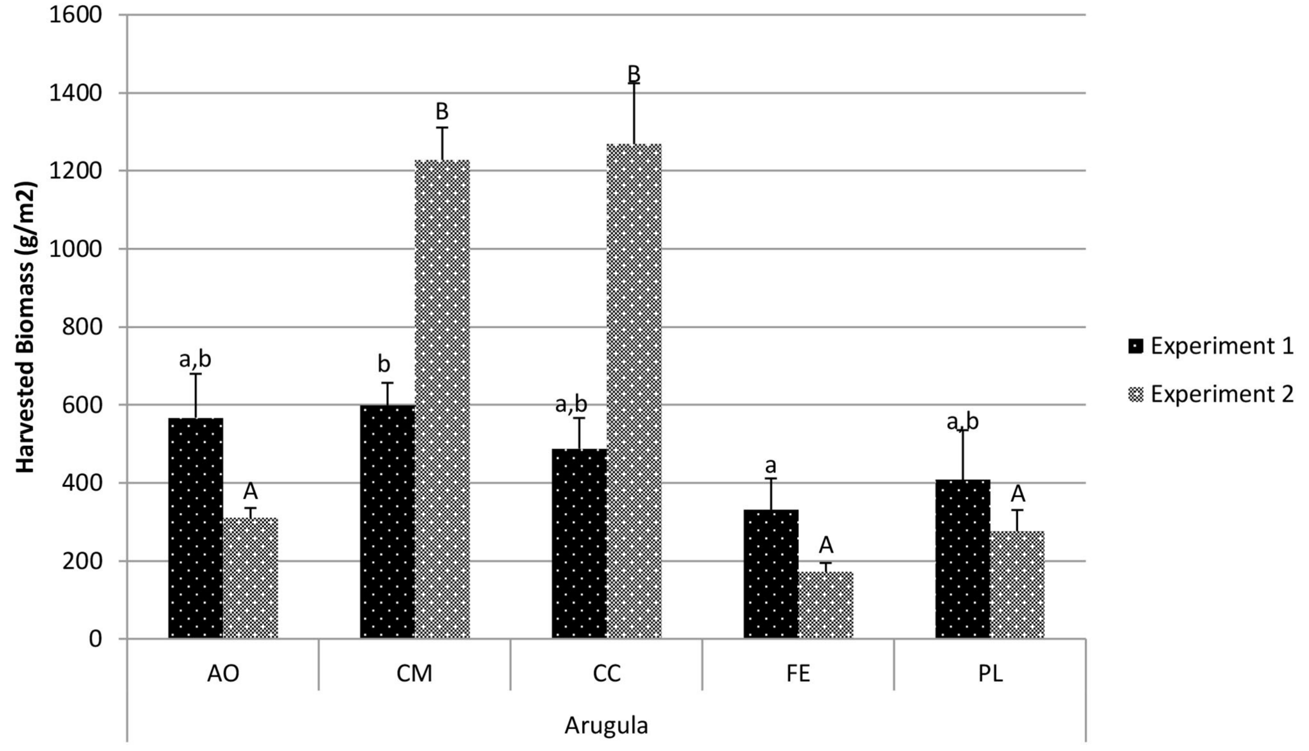

FIGURE 1 | Comparison of arugula harvested biomass between treatments in experiment 1 and experiment 2. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

all similar to one another. In experiment 2, the CC and CM treatments resulted in significantly greater harvested biomass than the AO, FE, and PL treatments ($p < 0.01$).

The harvested biomass for mizuna (**Figure 2**) in experiment 1 varied among treatments, and also varied among treatments in experiment 2. In experiment 1, the CM, CC, FE, and PL treatments all resulted in similar harvested weights, and biomass in the AO treatment was significantly greater than that in the other four treatments (1,719.6 g/m^2; $p < 0.001$). In experiment 2, the biomass with the CM treatment yielded a higher harvest biomass than the other four treatments (2,087 g/m^2; $p < 0.01$).

The harvested biomass for red giant mustard (**Figure 3**) was highest in experiment 1 for the AO treatment (1,314.3 g/m^2) and was significantly higher than all other treatments in experiment 1 ($p < 0.002$). Yield in the CC, FE, and PL treatments were all statistically similar to one another, while the CM treatment resulted in significantly lower yields than these three treatments (303.6 g/m^2; $p < 0.01$). In experiment 2 the yield with the CM treatment had the highest harvested biomass (1,747.3 g/m^2) and was statistically similar to the AO treatment (1,435.2 g/m^2; $p = 0.22$) and the CC treatment (1,343.2 g/m^2; $p = 0.081$). The PL treatment resulted in the lowest harvested biomass (205.0 g/m^2) and was significantly lower than all other treatments ($p < 0.001$).

Harvested spinach biomass for experiment 1 (**Figure 4**) differed among treatments. The AO treatment (1,161.5 g/m^2) was similar to the CC treatment (834.6 g/m^2; $p = 0.392$), but significantly higher than the CM, FE, and PL treatments ($p < 0.05$). The CM treatment had the lowest overall harvested biomass (418.6 g/m^2), but was statistically similar to the CC, FE and PL treatments. In experiment 2, the AO resulted in the highest overall harvested biomass (1,494.1 g/m^2) and was statistically similar to the CM treatment (1,195.8 g/m^2; $p = 0.376$) and the CC treatment (996.4 g/m^2; $p = 0.058$).

Minerals

In experiment 1, the mineral concentration in the harvested plants varied greatly among treatments and species (**Table 3**). For red giant mustard, there was no treatment difference for iron, potassium and sodium concentrations. For calcium, the CC resulted in the highest concentration (191.3 mg/100 g fw), which was statistically higher than the CM treatment (138.2 mg/100 g fw; $p = 0.031$), while the FE, AO and PL treatments were all similar. For the mizuna plants the potassium and sodium concentrations were most affected by treatment. For potassium, the CM treatment (860.3 mg/100 g fw) was significantly higher than the CC (393.1 mg/100 g fw), FE (120.16 mg/100 g fw), and PL (164.39 mg/100 g fw) treatments ($p < 0.001$). Sodium concentrations were also greatly affected by the treatments in the mizuna plants with nearly a 10-fold difference between the CM treatment (14.25 mg/100 g fw) and the FE treatment (142.66 mg/100 g fw; $p < 0.001$). Statistical relations between the collected soil and tissue mineral data in experiment 1 showed a

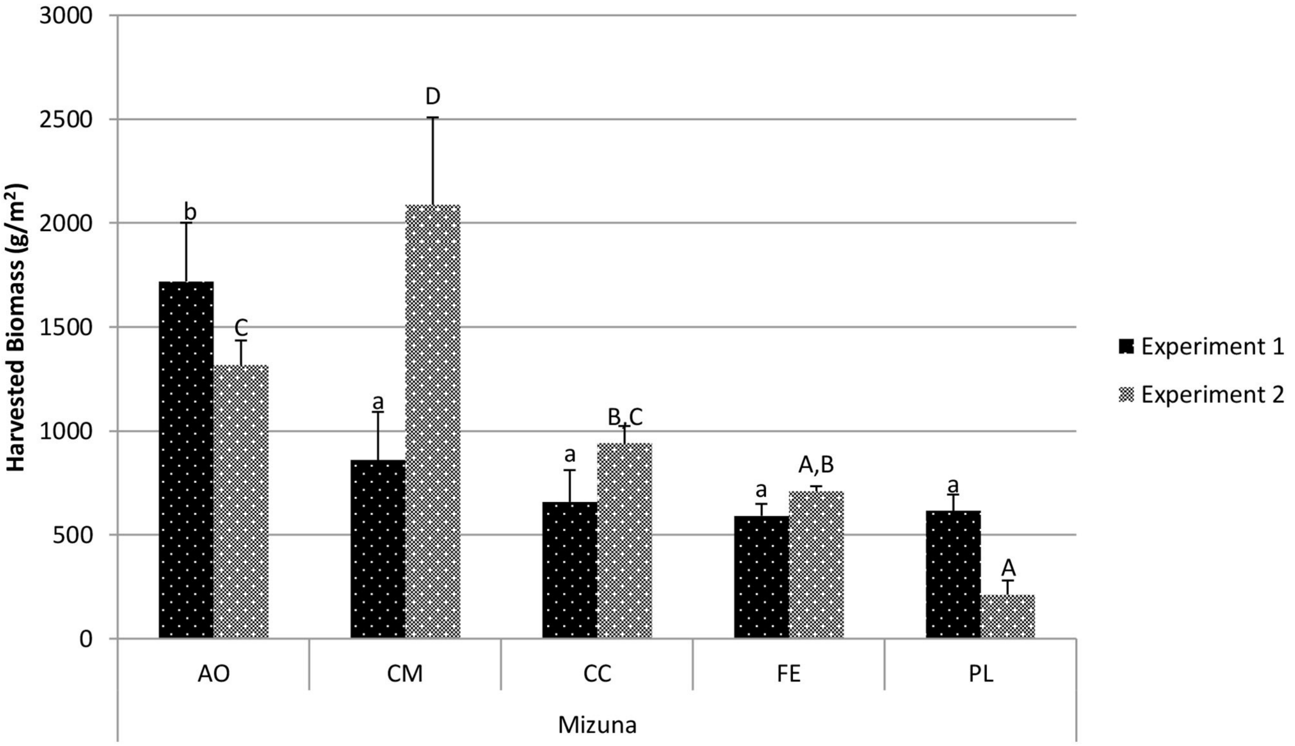

FIGURE 2 | Comparison of mizuna harvested biomass between treatments in experiment 1 and experiment 2. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

significant correlation between potassium concentrations in the collected mizuna plants and soil at time of harvest ($p = 0.028$), and a positive correlation for mizuna and red giant mustard (soil mineral data only collected for experiment 1).

Vitamin C

Mizuna vitamin C was analyzed for all five fertility treatments in experiment 1 and for four fertility treatments for experiment 2. In experiment 1, the PL treatment resulted in the highest average tissue vitamin C concentration (16.1 mg/100 g fw), but was statistically similar to all other treatments, except for the CM treatment ($p = 0.002$; **Figure 5**). In experiment 2, the CC treatment resulted in the highest vitamin C concentration (48.13 mg/100 g fw) compared to all other treatments ($p < 0.001$; **Figure 5**). Tissue vitamin C concentration in plants grown in CM was higher than FE (16.86 mg/100 g fw; $p = 0.018$) and AO (16.03 mg/100 g fw; $p = 0.008$). Between experiments, fertility treatments resulted in a wide fluctuation in mizuna vitamin C concentrations, with all treatment averages lower in the first experiment. For the CC treatment, the average tissue vitamin C for experiment 1 was 11.94 mg/100 g fw whereas in experiment 2 it was 48.13 mg/100 g fw.

In experiment 1, the red giant mustard PL treatment resulted in the highest mean vitamin C concentration (21.26 mg/100 g fw), but was statistically similar to the CC treatment (15.7 mg/100 g fw; $p = 0.116$) (**Figure 6**). The PL treatment resulted in significantly higher vitamin C concentrations than the FE treatment (14.03 mg/100 g fw; $p = 0.049$), AO treatment (12.48 mg/100 g fw; $p = 0.022$) and CM treatment (8.45 mg/100 g fw; $p = 0.022$). In experiment 2 (**Figure 6**), the CC treatment (32.4 mg/100 g fw) resulted in similar vitamin C concentrations to the FE treatment (27.53 mg/100 g fw; $p = 0.369$), the AO treatment (43.23 mg/100 g fw; $p = 0.067$) and CM treatment (23.36 mg/100 g fw; $p = 0.115$).

Fiber

For mizuna plants in experiment 1 (**Figure 7**), the CC treatment (2.94 g/100 g fw) resulted in statistically similar concentrations of total dietary fiber compared to the PL treatment (3.24 g/100 g fw; $p = 0.051$) and also to the FE treatment (3.06 g/100 g fw; $p = 0.367$). Plants grown in both the CM treatment (2.04 g/100 g fw) and the AO treatment (1.82 g/100 g fw) had similar total dietary fiber concentrations, $p < 0.001$ and $p < 0.001$, respectively. In experiment 2, the CC treatment (4.30 g/100 g fw) resulted in significantly higher total dietary fiber than the CM treatment (2.47 g/100 g fw; $p < 0.001$) and lower than the AO treatment (3.77 g/100 g fw; $p = 0.02$) and the FE treatment (4.96 g/100 g fw; $p = 0.007$).

For red giant mustard plants in experiment 1 (**Figure 8**), the CC treatment (2.88 g/100 g fw) resulted in statistically similar total dietary fiber concentrations compared to the PL treatment (2.86 g/100 g fw; $p = 0.868$). In contrast, the CM treatment

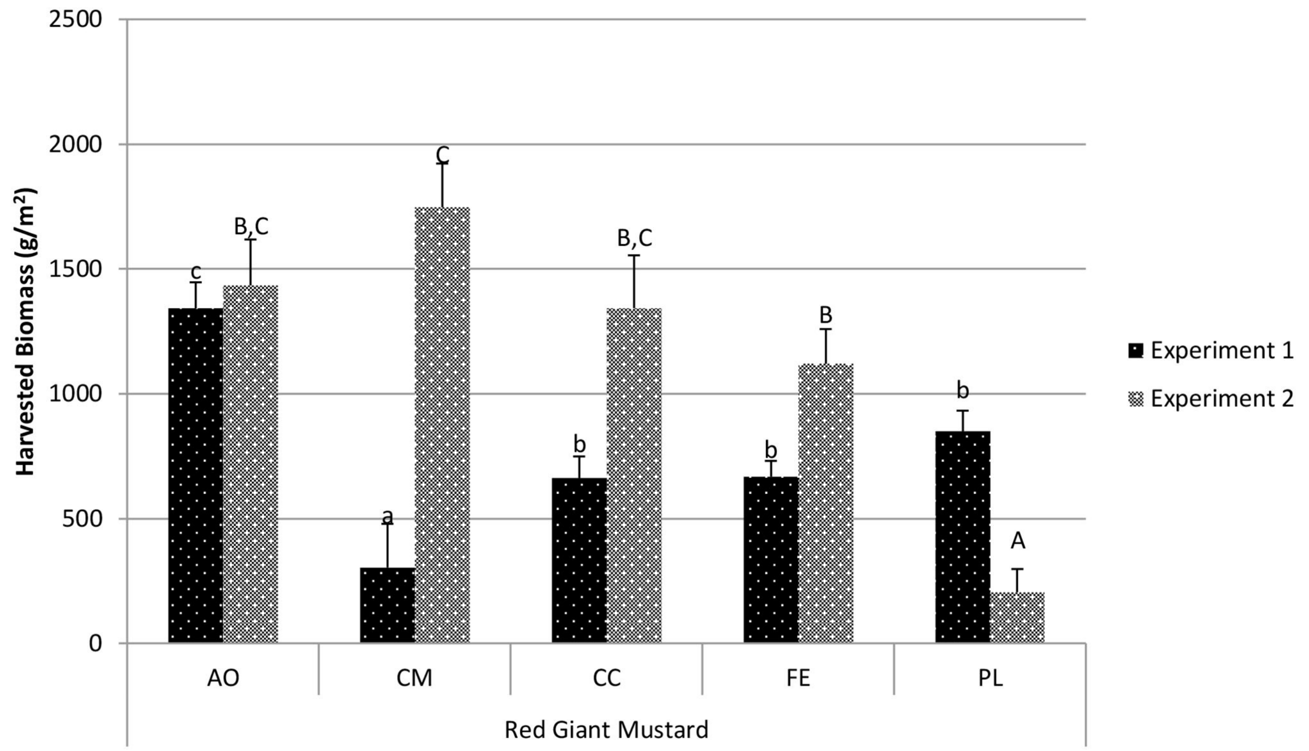

FIGURE 3 | Comparison of red giant mustard harvested biomass between treatments in experiment 1 and experiment 2. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

(2.03 g/100 g fw; $p < 0.001$), the FE treatment (2.20 g/100 g fw; $p < 0.001$) and AO treatment (1.44 g/100 g fw; $p < 0.001$) produced plants with significantly less dietary fiber than the CC treatment. In experiment 2, total dietary fiber concentrations in plants grown in the CC treatment (4.79 g/100 g fw) were statistically similar to both the FE treatment (4.65 g/100 g fw; $p = 0.588$) and the AO treatment (4.32 g/100 g fw; $p = 0.091$), but were greater than the CM treatment (2.01 g/100 g fw; $p < 0.001$).

Nitrate-N

Compared to the four other treatments, the plants grown in the CM treatment had significantly higher nitrate-N concentrations than all other treatments ($p < 0.01$; **Table 4**). For the arugula in experiment 1, tissue nitrate-N concentrations in the CM treatment [1,244 ppm (mg/kg fresh weight)] and the AO treatment (1,255 ppm) were significantly higher than in the other three treatments ($p < 0.001$). In the mizuna plants, tissue nitrate concentrations in the CM (1,443 ppm) and AO (1,656 ppm) treatments were significantly higher than the other treatments ($p < 0.001$). In experiment 2, arugula had the same nitrate-N concentration for the CC, FE, and PL treatments, while tissue nitrate-N in the CM treatment (734 ppm) was significantly higher than the rest ($p < 0.001$). For the spinach, mizuna and red giant mustard plants analyzed in experiment 2, the CM treatment resulted in the highest tissue nitrate-N concentration ($p < 0.001$). Statistical relations between the soil nitrate-N concentrations (taken at first harvest) and plant tissue nitrate-N concentrations showed a significant correlation ($p < 0.05$) for arugula, mizuna and red giant mustard plants analyzed from experiment 1 (**Table 5**).

Comparison With Plants Grown in Commercial Greenhouses

Red giant mustard and mizuna plants were collected from three commercial greenhouses in Minnesota for shortfall nutrients and mineral comparison throughout experiment 1 and experiment 2 (**Table 6**). The red giant mustard vitamin C concentrations in experiment 2 nearly all fell within range of produce grown in commercial greenhouses, while for experiment 1 vitamin C was slightly lower than in the commercial greenhouses. Calcium levels in the commercial produce were higher than both experiment 1 and 2 for red giant mustard. For mizuna, experiment 1 had slightly lower vitamin C concentrations than the comparison produce, while experiment 2 had similar concentrations. Similar to red giant mustard, the mizuna plants also had much lower calcium concentrations in experiments 1 and 2 (128–251 mg/100 g) compared to the comparison mizuna (229–422 mg/100 g). The average concentration in the commercial comparison red giant mustard was 1,599 ppm, which was less than the CM and AO treatments in experiment 1, but greater than all other red giant mustard plants analyzed. The commercial comparison mizuna had an average concentration

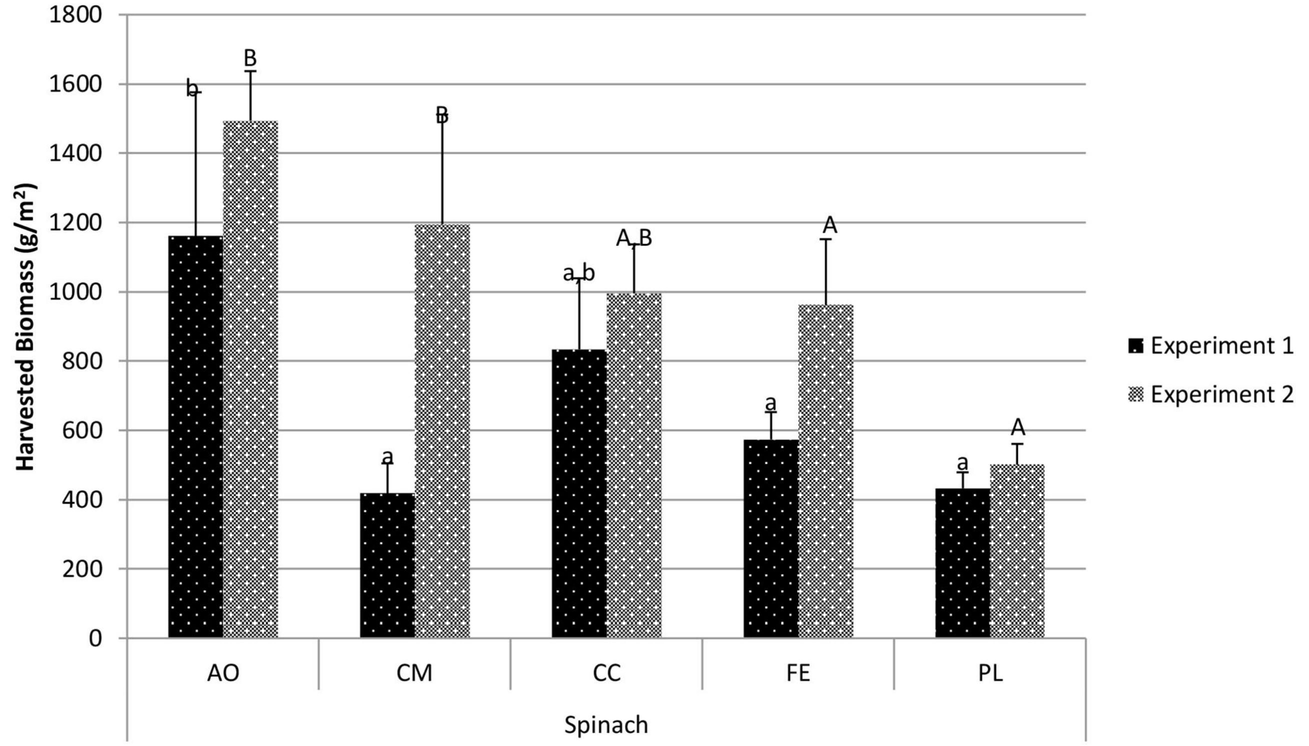

FIGURE 4 | Comparison of spinach harvested biomass between treatments in experiment 1 and experiment 2. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

TABLE 3 | Mineral comparison between fertility treatments (mg/100 g fresh weight)—experiment 1.

		Fertility treatment				
	Mineral	**CC**	**CM**	**FE**	**AO**	**PL**
Red giant mustard	Ca	191.3^{b}	138.2^{a}	$166.1^{a,b}$	$188.3^{a,b}$	$189.4^{a,b}$
	Fe	1.1	0.7	1.1	0.7	1.2
	K	416	600.54	347.93	813.76	262.60
	Na	32.3	43.7	144.9	55.9	75.8
Mizuna	Ca	239.3^{b}	128.9^{a}	201.6^{b}	229.6^{b}	205.9^{b}
	Fe	0.6^{a}	0.9^{b}	0.6^{a}	$0.8^{a,b}$	0.7^{a}
	K	393.1^{b}	860.2^{c}	120.1^{a}	750.4^{c}	164.4^{a}
	Na	26.4^{a}	14.3^{a}	142.7^{d}	70.7^{b}	105.2^{c}
Arugula	Ca	200.9^{b}	$130.7^{a,b}$	$154.62^{a,b}$	$187.8^{a,b}$	112.9^{a}
	Fe	0.9	0.8	0.8	0.7	0.9
	K	455.3^{b}	950.7^{d}	$309.0^{a,b}$	662.7^{c}	277.6^{a}
	Na	26.6^{a}	10.8^{a}	113.7^{b}	31.4^{a}	$60.7^{a,b}$
Spinach	Ca	83.9	*	144.3	*	76.3
	Fe	0.8	*	0.9	*	0.8
	K	995.1^{b}	*	287.4^{a}	*	820.2^{b}
	Na	42.8	*	137.9	*	92.1

Data shown are mean (n = 3) for each respective species. Results analyzed using ANOVA Tukey HSD for significance testing (p <0.05). Significance analyzed for each species within each experiment.

**Indicates missing samples due to space capacity of experiment in greenhouse.*

a,b,c,d indicate means within a row followed by the same letter are not significantly different at p < 0.05.

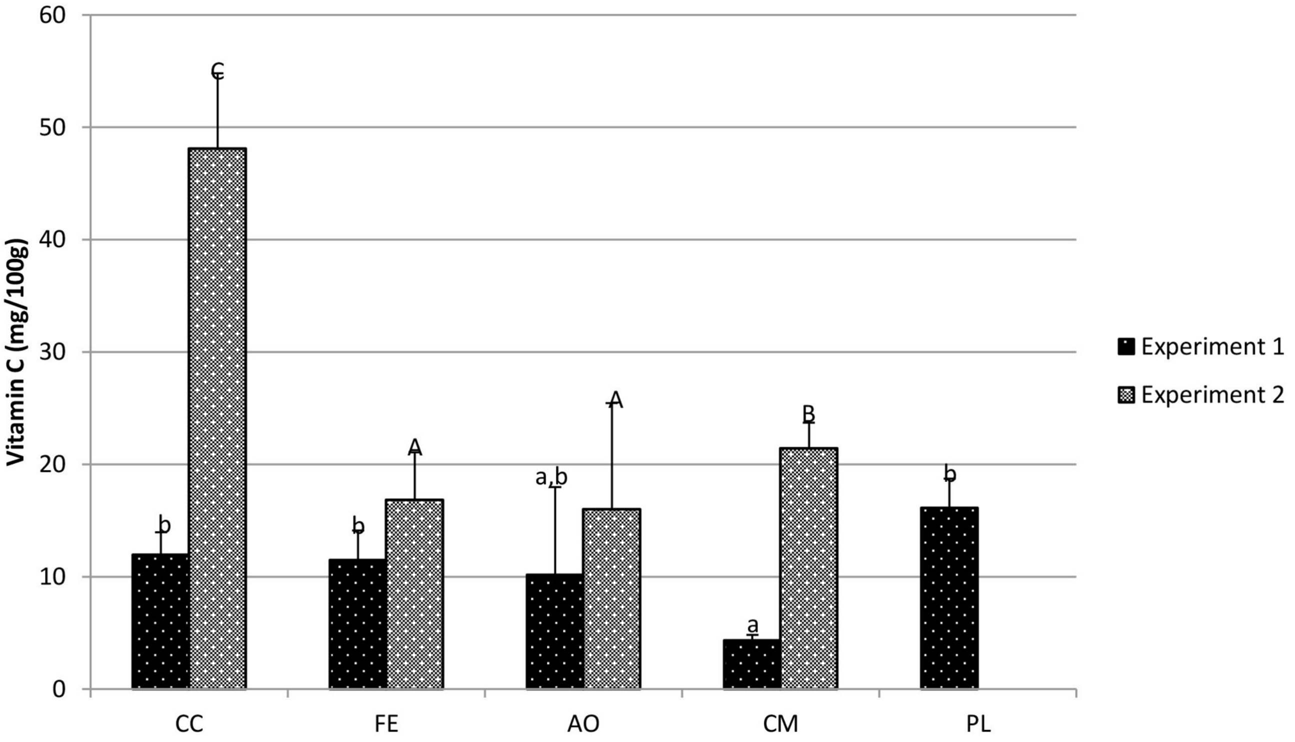

FIGURE 5 | Mizuna vitamin c concentration (mg/100 g fresh weight) in harvested biomass—experiment 1 and experiment 2. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

of 1,175 ppm, which was only less than the CM and AO treatments in experiment 1, and greater than all other mizuna plants analyzed, which was the same trend for both species of plants analyzed.

DISCUSSION

Minerals

For the arugula, red giant mustard, mizuna and spinach plants evaluated in these experiments, significant differences in iron, potassium, calcium, sodium, vitamin C, and fiber between the fertility treatments were dependent on the experiment. Changes in any of these shortfall nutrients or nutrients of high interest depended on the season in which the plants were grown. Calcium concentrations were most likely higher in the CC treatment plants because the CC treatment had the second highest calcium concentration of all the media analyzed.

Under the FDA requirements for labeling, the minimum requirement of calcium in a product needed to make a "good source" nutrient content claim is 130 mg per 100 g serving (10% of 1,300 mg required by DV), nearly all of the plants under all the fertility treatments would qualify from both experiments. For potassium, a "good source" claim would require a minimum of 470 mg per 100 g serving. This claim could only be made for the CM and AO treatments (based on averages from both experiments) in the red giant mustard, mizuna and arugula plants analyzed.

The use of different fertility treatments affects potassium which is relevant as potassium is a nutrient of concern and now required on the Nutrition Facts panel. The FE treatment produced the highest average sodium concentrations in all plants analyzed in experiments 1 and 2 (with the exception of arugula in experiment 2). Dietary recommendations support lowering sodium intake, but the amount of sodium in the greens is universally low.

Few other studies have been published on the mineral content of vegetables grown by organic vs. conventional growing methods. Trace minerals were measured in the five most-consumed vegetables in the US, potato, lettuce, tomato, carrot, and onion for conventional vs. organic production (Hadayat et al., 2018). The vegetables were collected at grocery stores and not produced in a greenhouse or research plot. They reported little differences in content of trace minerals between conventional and organic vegetables. Most of the interest in mineral content of organic crops is based on lack of synthetic pesticides and mineral fertilizers in organic production.

Vitamins

Vitamin C is a highly unstable water-soluble vitamin known for its antioxidant properties, both in humans and plants. Key effects on vitamin C concentration include the growing conditions,

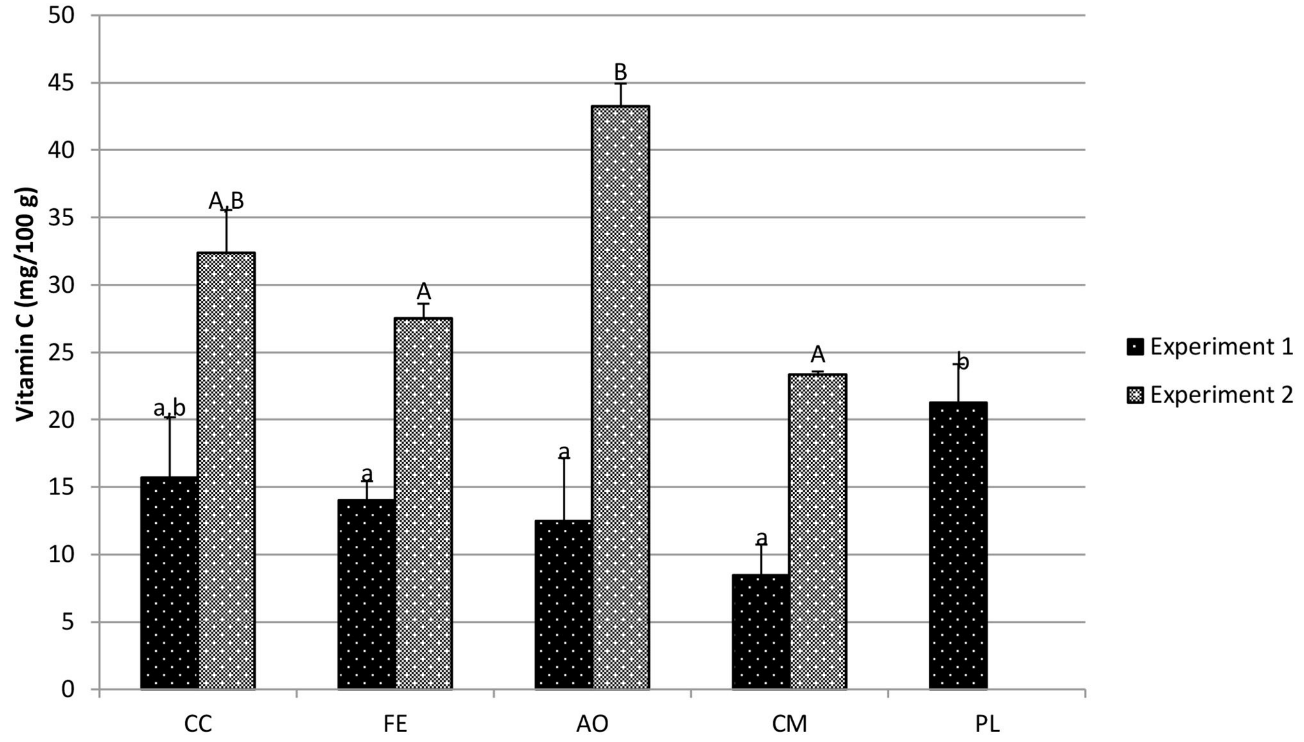

FIGURE 6 | Red giant mustard vitamin c concentration (mg/100 g fresh weight) in harvested biomass—experiment 1 and experiment 2. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

plant stage at harvest, storage temperature and wide range of postharvest conditions (Phillips et al., 2018). Traditionally, accumulation of vitamin C is increased whenever plants are exposed to high oxidative stress, including full sunlight, low nitrogen availability in the soil and drought conditions (Kaack et al., 2001). For fresh produce, vitamin C losses can be enhanced when postharvest storage is extended, or at higher temperature, low relative humidity and freezing. High nitrogen fertilizers have also been associated with decreased vitamin C concentrations in many fruits and vegetables (Lee and Kader, 2000).

For all treatments in both experiments, for both mizuna and red giant mustard plants, plants harvested in experiment 2 had significantly higher vitamin C concentrations than in experiment 1 ($p < 0.001$). Plants in experiment 2 were grown in early spring, whereas plants grown in experiment 1 were grown during the winter months. Consequently, fluctuations in vitamin C concentrations between experiments were most likely due to changes in sunlight exposure. Among all the combined samples analyzed, the CC treatment had the highest vitamin C concentrations ($p = 0.02$) compared with the other treatments, and equal or higher calcium concentrations in those analyzed plants, although only in experiment 1.

Many pre-harvest factors (genotypic, climatic and environmental) influence vitamin C; thus, variation between experiments and growing seasons is common (Phillips et al., 2018).

Fiber

Dietary fiber is a mixture of complex organic substances that are non-digestible in the upper gastrointestinal tract, present as both soluble and insoluble compounds. Studies have analyzed dietary fiber differences between conventionally and organically grown plums, with little differences observed (Lombardi-Boccia et al., 2004). To our knowledge, dietary fiber has not been analyzed in nutrient dense crops that address shortfall nutrients, comparing either organic or conventional nutrient sources.

Under the 2016 FDA Nutrition and Supplements Facts Label Revisions, the new DV for dietary fiber is increasing to 28 g/d. Based on this new rule, 2.8 g fiber/serving will be required to make a "good source" nutrient content claim. Based on the combined averages from both experiments, only the mizuna and red giant mustard with the CC and FE treatments would qualify for this claim. Based on a single experiment, the mizuna and red giant mustard with the PL treatment would also qualify.

Nitrate-N

Nitrate-N concentrations varied greatly among treatments, species and experiments. Additionally, the significant correlation ($p < 0.05$) between plant tissue nitrate-N concentrations in arugula, mizuna and red giant mustard from experiment 1, and soil nitrate-N concentrations, showed that high nitrate-N in soil leads to high nitrate-N in harvested plant tissue.

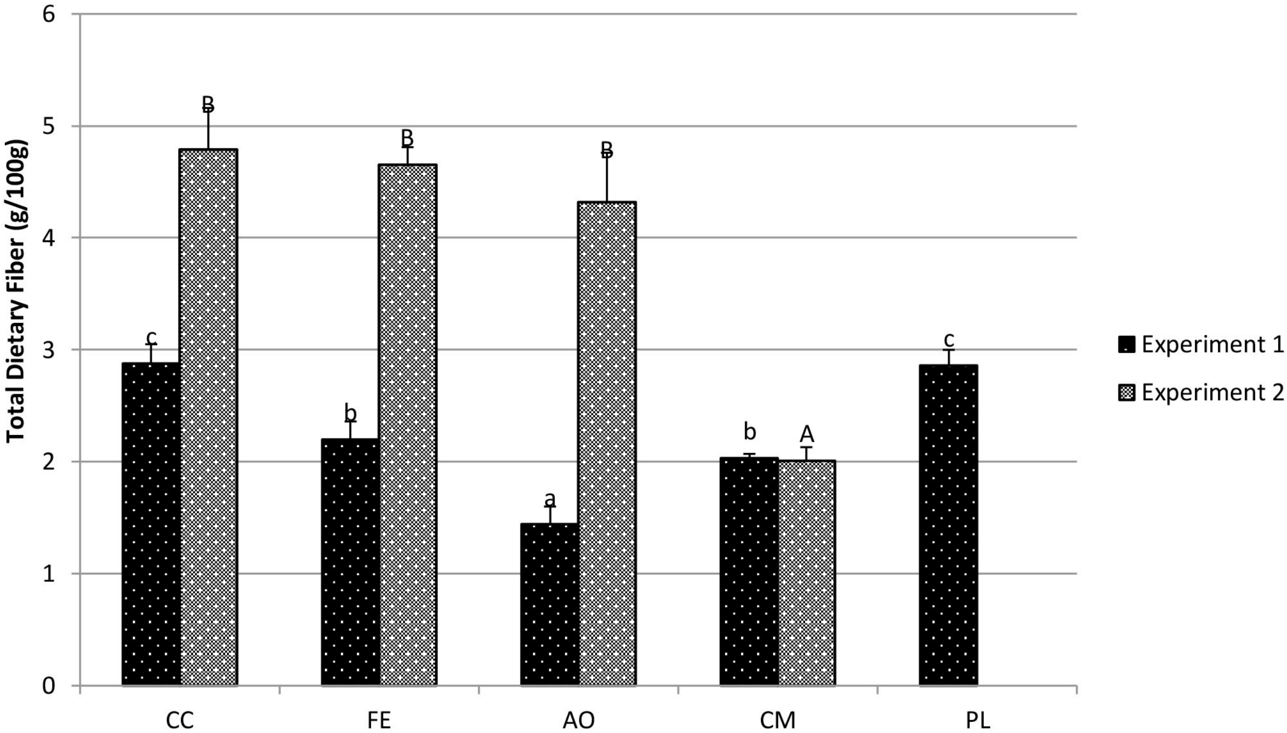

FIGURE 7 | Total dietary fiber in mizuna (g/100 g fresh weight) determined with AOAC 991.43. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

Reviews of differences between organic and conventionally grown produce is that variables shared alike by organic and conventional produce during production, harvest, and postharvest handling and storage are not applied (Lester and Saftner, 2011). Therefore, many reviews find no evidence of a difference in nutrient quality between organically and conventionally produced foodstuffs (Dangour et al., 2009). More recent reviews and meta-analyses report that organic crops, on average, have higher concentrations of antioxidants and lower concentrations of pesticide residues than the non-organic comparators across regions and production seasons (Baranski et al., 2014). The authors note that a main challenge to interpreting comparisons of organic and inorganic food production systems is the high heterogeneity arising from combinations of crops, crop types, countries, and agronomic background conditions. In these analyses, heterogeneity is extremely high for most of the composition parameters for which significant differences are detected.

It has been estimated that 80% of the nitrate in people's diets comes from vegetable consumption (Karwowska and Kononiuk, 2020) as plants require bioavailable nitrogen to carry out photosynthesis for plant growth and development. Vegetables accumulate nitrates when the uptake of the nitrate ion exceeds its reduction and subsequent assimilation (Hakeem et al., 2017). This accumulation depends on numerous factors such as the composition of the soil where the plant is grown, the type of crop production, the type of fertilizer used, the season of the yar the crop is grown, and environmental conditions in which the crop is grown. Nitrate content also differs in plant species and varieties with generally leafy greens being concentrated in nitrates as they are good nitrate accumulators due to their rapid growth and that nitrates tend to accumulate in leaves and the leaves are what is consumed by consumers.

Very few differences were noted in nitrate concentrations of conventional and "organic" labeled vegetables taken from 5 U.S. metropolitan cities in a survey study sampling of retail outlets (Nunez de Gonzalez et al., 2015). However, they did report differences in nitrate content for some conventional and organic vegetables in different cities with the organic vegetables being lower in nitrate content. There are major research challenges comparing differences in nutrients and nitrates resulting from farming practices as studies have used different experimental designs and been carried out in different regions and growing seasons. An advantage of our research is that we studied vegetables known to be high in nutrients and nitrates, yet a disadvantage is that our studies were done in greenhouses, which allows us to control some variables, yet we did not control growing season, likely a critical variable in nitrate accumulation.

For nearly half of the produce analyzed in this study, an average consumer (68 kg) would exceed their recommended daily nitrate intake with only two servings (100 g/serving) of produce. The European Food Safety Authority (EFSA) and The World

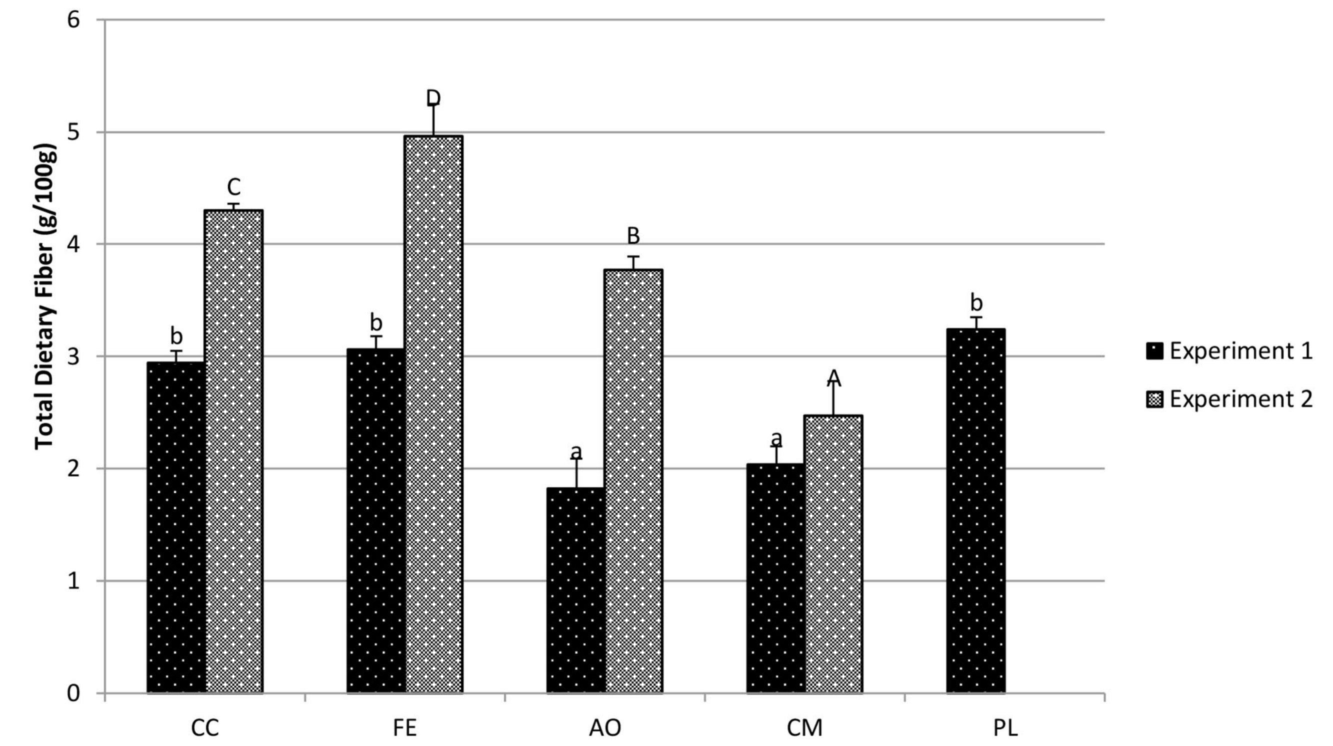

FIGURE 8 | Total dietary fiber in red giant mustard (g/100 g fresh weight) determined with AOAC 991.43. Data shown are mean ± standard deviation for two different replicate experiments. Significant differences among mean values are indicated with different letters above bars ($p < 0.05$) with lower-case letters for experiment 1 and capital letters for experiment 2. Results analyzed using ANOVA Tukey HSD for significance testing.

TABLE 4 | Nitrate-N concentrations (NO_3-N ppm; mg/kg fresh weight) in arugula, spinach, mizuna and red giant mustard for experiment 1 and experiment 2.

		Fertility treatments				
		CC	**CM**	**FE**	**AO**	**PL**
Experiment 1	Arugula	266[a]	1,244[b]	218[a]	1,255[b]	100[a]
	Spinach	1,988[b]	N/A	1,236[a,b]	N/A	932[a]
	Mizuna	171[a]	1,443[b]	20[a]	1,656[b]	12[a]
	Red Giant Mustard	171[a]	1,673[b]	194[a]	2,279[c]	21[a]
Experiment 2	Arugula	5[a]	734[b]	4[a]	N/A	6[a]
	Spinach	587[c]	1,251[d]	241[a,b]	473[b,c]	48[a]
	Mizuna	3[a]	614[b]	<1[a]	2[a]	<1[a]
	Red Giant Mustard	19[a]	877[b]	3[a]	16[a]	3[a]

a,b,c,d indicate means within a row followed by the same letter are not significantly different at $p < 0.05$.
N/A, sample not available.

Health Association (WHO) recommend that daily nitrate intake be below 3.7 mg dietary nitrate/kg of body weight, as expressed by the Acceptable Daily Intake (ADI). When converted from nitrate to NO_3-N as used in this study (multiplying by 0.226) this would be equal to a recommendation 50 mg, or less, of calculated NO_3-N for a 60 kg individual). Vegetables are the number one source of nitrates in the diet for many populations, so monitoring intake is crucial to minimize the potentially harmful effects of high nitrate diets (EFSA, 2008; Hord et al., 2009). Careful considerations need to be made when applying nitrogen to both conventional and organic plants, as excessive nitrogen yields excessive amounts of nitrate accumulation in plant tissues. Both organic and conventional produce can have excessive amounts of nitrates, depending on growing and environmental conditions.

CONCLUSION

Organic vs. conventional growing practices in greenhouse grown leafy greens impacted amounts of Vitamin C and potassium levels in analyzed plant tissue. No consistent differences were found for fiber, calcium, iron and sodium concentrations in the tissue analyzed. Nitrate levels were generally higher in conventionally grown greens, although other factors including growing season were found to influence nitrate levels. Consumer demand for organic production of greens supports that organically grown leafy greens provide important short fall nutrients and a likely lower in nitrate than conventionally grown greens from greenhouses.

Limitations

Comparing nutrient differences in organic vs. conventionally grown crops is incredibly difficult. One limitation of such studies is that food grown in organic systems may consist of more dry matter than conventionally-grown food. If this is in fact the case, using dry weight would not accurately compare nutrient levels between the different production systems. Fresh weight may be a more accurate measure.

A second limitation relates to the study publication source. Guéguen and Pascal note that independent reviews have not been finding significant differences between organically grown and conventionally grown foods, whereas reviews conducted by organic agricultural organizations skew toward the positives of organic farming (Guéguen and Pascal, 2013). Organic agricultural production systems have benefits beyond nutrient levels or lower nitrate levels, yet it is difficult to systematically study these advantages in traditional research designs.

A third limitation is study design. This could encompass the general design of the study, incomplete reporting of study details, lack of statistical interpretation, or lack of control of growing conditions in retail-bought foods (Guéguen and Pascal, 2013). These issues make it difficult to interpret study outcomes in useful ways. Because our research was conducted in greenhouses, we had more control of growing conditions. Yet the most practical research for consumers is the nutrient and nitrate levels of the foods they purchase at the retail market. These studies are confounded as the organic and conventional foods they purchase come from different fields in different countries and the vegetables may have been in the market for days or weeks before being purchased and consumer.

TABLE 5 | Soil nitrate-N concentrations (NO_3-N ppm; mg/kg dry weight) correlated to plant tissue nitrate-N concentrations (NO_3-N ppm; mg/kg fresh weight) in arugula, mizuna and red giant mustard in experiment 1.

	Pearson's *r*	*P*
Arugula	0.813	<0.001
Mizuna	0.539	0.047
Red giant mustard	0.723	0.002

Statistical relations were determined with bivariate correlations (Pearson's r), and a $p < 0.05$ was accepted as statistically significant. Correlations were made between soil and tissue Nitrate-N concentrations for each individual plant species.

Future Research

Different agricultural management systems may have an impact on the sustainability of food systems and affect human health, food security, and environmental sustainability (Schulz and Slavin, 2021). For different crops, the impact of organic production on nutrient values will vary greatly. Thus, it is not surprising that there is little clear nutritional advantage of consumption of organic foods when reviews of the literature are published.

Our study was an attempt to combine research teams in horticulture, soil science, food science, and nutrition to answer a practical question, are there differences in nutrient and nitrate content when we compare organic vs. conventional growing systems for leafy greens. The producers of leafy greens in deep winter greenhouses in Minnesota were the stakeholders that wanted practical advice on how to grow organic leafy greens in greenhouses and agreed to contribute their produce to determine if results from commercial operations would vary from a research greenhouse. The data on biomass of leafy green were based on the practical need of growers to be sure that changing growing conditions would not lower yield in their product.

Costs for nutrient analysis limit research in this field and we chose our nutrients to measure as ones that are under consumed by the US population and that have health benefits. Our results suggest that factors such as sunlight, other growing conditions, time of the year, are more important on content of Vitamin C than organic vs. conventional growing conditions.

Unlike essential nutrients like vitamins, minerals, and dietary fiber that are known to be beneficial for human health, other compounds like nitrate are associated with health risks. The largest amount of nitrates is accumulated in plants growing in a nitrate-rich environment and the most important sources of

TABLE 6 | Conventionally grown produce collected throughout both experiments from three Minnesota growers and greenhouses.

	Vitamin C (mg/100 g)	Fiber (g/100 g)	Ca (mg/100 g)	Fe (mg/100 g)	K (mg/100 g)	Na (mg/100 g)	Nitrate-N (ppm)
Red giant mustard							
Mean*	31.73	3.81	251.6	0.76	558.3	48.9	1,599
Median	29	3.5	254.3	0.79	585.5	42.8	2,218
Range	20.4–45.8	2.19–5.74	254–331	0.64–0.83	486–602	33–71	59.7–2,520
Mizuna							
Mean*	28.6	3.65	294.8	0.87	482.9	42.1	1,175
Median	23.4	3.5	232.3	0.93	492.6	38.4	1,577
Range	16.6–45.8	2.13–5.34	229–422	0.72–0.97	395–560	36–51	70–1,878

**Data displayed are three triplicate samples from three different greenhouses and growers.*

dietary intake of nitrate are vegetables and fruits (Karwowska and Kononiuk, 2020). Application of fertilizers, nitrate reductase activity, growth rate and growth conditions, including intensity of light, level of rainfall, significantly affect the nitrate content of vegetables. Leafy green vegetables have higher levels of nitrates compared to seeds or tubers. Lettuce and spinach have the highest nitrate content and the content of nitrates in vegetables is strongly influenced by seasonality and the cultivation system (Lucarini et al., 2012). They reported that lettuce biodynamically grown accumulated 1.3–2 times less nitrate than the respective organically grown plants. Our nitrate results likely show differences in time of season more than organic vs. conventional growing conditions. Other research supports that organic production of leafy green vegetables may lower the nitrate content of the vegetables compared to conventional production, if other variables such as seasonality and production systems are controlled.

Another systemic factor is the difference in nutrient and compound concentrations between a full, open-field organic system following all organic principles and practices, and a limited greenhouse system operating under fewer organic principles and practices. The key question is: how, and to what degree, do nutrient and compound concentrations differ between these two types of systems?

Future research must consider that reasons for adoption of organic growing conditions extend far beyond nutrition. Support for local food production and other societal variables must be considered when analyzing advantages to organic food production. And of course, the economics of organic food production are an overlay that should be considered in future research. If producers are guaranteed a higher price for their organic foods, then it makes sense to invest in organic growing systems on their farm. A fair economic system would ensure the producer a higher price for their product. And this would translate into higher prices for organic foods in the marketplace, which bring about issues of social justice and food security. Research in food systems requires teamwork across agriculture, the government, basic and social scientists across disciplines and of course economists.

In summary, this novel study investigated the effects of organic soil amendments upon nutrients and nitrate, in leafy greens. These soil amendments significantly affected vitamin C, nitrate, and potassium concentrations, although results support that other factors, including seasonality and sunlight were important factors determining the level of these nutrients in organic vs. conventional leafy greens. The treatments did not significantly affect fiber, calcium, iron, or sodium. The various treatment responses support the complexity of determining nutrient content of foods grown by organic and conventional growing practices and the reasons for the limited research data that organic food production is always a win for nutrient content.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JE was the Principal Investigator for the grant. JS and CR were the co-investigators. JG and MR supervised graduate students in plant sciences who worked on the project. JC was a graduate student in Food Science while LP was a graduate student in Horticulture. The study was designed by the PIs and all investigators were involved in conducting the study, sample analysis, and data entry. JC wrote the first draft of this publication and ES revised the manuscript as part of her MS in Nutrition at the University of Minnesota. All authors have reviewed, revised, and approved the manuscript.

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REFERENCES

Baranski, M, Srednicka-Tober D., Volakakis, N., Seal, C., Sanderson, R., Stewart, G.B., Benbrook, C., et al. (2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analysis. *Br. J Nutr*. 112, 794–811. doi: 10.1017/S0007114514001366

Dangour, A. D., Dodhia, S. K., Hayter, A., Allen, E., Lock, K., and Uauy, R. (2009). Nutritional quality or organic foods: a systematic review. *Am. J. Clin. Nutr*. 90, 680–685. doi: 10.3945/ajcn.2009.28041

Donaldson, D. (2021). *Organic Market Summary and Trends*. Available online at: https://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture/organic-market-summary-and-trends/ (accessed July 27, 2021).

EFSA (2008). Panel on contaminants in the food chain nitrate in vegetables—scientific opinion of the panel on contaminants in the food chain. *EFSA J*. 689, 1–79. doi: 10.2903/j.efsa.2008.689

Funk, C., and Kennedy, B. (2016). *Americans' Views About and Consumption of Organic Foods*. Available online at: https://www.pewresearch.org/science/2016/12/01/americans-views-about-andconsumption-of-organic-foods/ (accessed July 17, 2021).

Grafton, K.B., Joern, A., Mallarino, D., Mengel, J., Dahl, D., Kaiser, D., et al. (2015). *Recommended Chemical Soil Test Procedures for the North Central Region (Publication No. 221)*. Available online at: https://extension.missouri.edu/

media/wysiwyg/Extensiondata/Pub/pdf/specialb/sb1001.pdf (accessed June 30, 2017).

Guéguen, L., and Pascal, G. (2013). "Organic foods," in *Encyclopedia of Human Nutrition Vol 3, 3rd Edn. editor*. B. Caballero (Amsterdam: Academic Press), 413–416.

Hadayat, N., De Oliveira, L.M., Da Silva, E., Han, L., Hussain, M., Liu, X., et al. (2018). Assessment of trace metals in five most-consumed vegetables in the US: conventional vs. organic. *Envirn. Poll.* 243, 292–300. doi: 10.1016/j.envpol.2018.08.065

Hakeem, K. R., Sabir, M., Ozturk, M., Akhtar, M. S., and Ibrahim, F.H. (2017). Nitrate and nitrogen oxides: sources, health effects and their remediation. *Rev. Environ. Contam. Toxicol*. 242, 183–217. doi: 10.1007/398_2016_11

Hamdard, J., Nagar, H., Gairola, S., Umar, S., and Suryapaini, S. (2009). Nitrate accumulation, growth and leaf quality of Spinach beet (Beta vulgaris Linn) as affected by NPK fertilization with special reference to potassium. *Indian J. Sci. Technol*. 2, 35–40. doi: 10.17485/ijst/2009/v2i2.2

Hord, N. G., Tang, Y., and Bryan, N. S. (2009). Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am. J. Clin. Nutr.* 90, 1–10. doi: 10.3945/ajcn.2008.27131

Hunter, D., Foster, M., McArthur, J. O., Ojha, R., Petocy, P., and Samman, S. (2011). Evaluation of the micronutrient composition of plant foods produced by organic and conventional agricultural methods. *Cr. Rev Food Sci. Nutr.* 51, 571–582. doi: 10.1080/10408391003721701

IFOAM-Organics International. (2020). *The Principle of Fairness*. Available online at: https://www.ifoam.bio/why-organic/principles-organic-agriculture/principle-fairness (accessed July 4, 2021).

Kaack, K., Nielsen, M., Christensen, L. P., and Thorup-Kristensen, K. (2001). Nutritionally important chemical constituents and yield of carrot (*Daucus carota L.*) roots grown organically using ten levels of green manure. *Acta Agric. Scand. Sect. B-Plant Soil Sci.* 51, 125–136.

Karnpanit, W., Benjapong, W., Srianujata, S., Rojroongwasinkul, N., Tanaviyutpakdee, P., Sakolkittinapakul, J., et al. (2018). Cultivation practice on nitrate, lead and cadmium contents of vegetables and potential health risks in children. *Int. J. Veg Sci.* 25, 512–528. doi: 10.1080/19315260.2018.1541952

Karwowska, M., and Kononiuk, A. (2020). Nitrates/nitrites in food – risk for nitrosative stress and benefits. *Antioxidants* 9, 241. doi: 10.3390/antiox9030241

Lee, S. K., and Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20, 207–220.

Leon, V. M., and Luzardo, O. P. (2020). Evaluation of nitrate contents in regulated and non-regulated leafy vegetables of high consumption in the Canarry Islands, Spain: risk assessment. *Food Chem. Toxicol*. 146, 111812. doi: 10.1016/j.fct.2020.111812

Lester, G. E., and Saftner, R. A. (2011). Organically versus conventionally grown produce: common production inputs, nutritional quality, and nitrogen delivery between the ttwo systems. *J. Agric. Food Chem*. 59, 10401–10406. doi: 10.1021/jf202385x

Lombardi-Boccia, G., Lucarini, M., Lanzi, S., Aguzzi, A., and Cappelloni, M. (2004). Nutrients and antioxidant molecules in yellow plums (*Prunus domestica L.*) from conventional and organic productions: a comparative study. *J. Agric. Food Chem.* 52, 90–94.

Lucarini, M., D'Evoli, L., Tufi, S., Garielli, P., Paoletti, S., Di Ferdinando, S., et al. (2012). Influence of growing system on nitrate accumulation in two varieties of lettuce and red radicchio of treviso. *J. Sci. Food Agric*. 92, 2796–2799. doi: 10.1002/jsfa.5526

Lucier, G., Allshouse, J., and Lin, B. (2004). *Factors Affecting Spinach Consumption in the United States*. Available online at: https://www.ers.usda.gov/publications/pub-details/?pubid=39496 (accessed June 30, 2017).

Mie, A., Andersen, H.R., Gunnarsson, S., Kahl, J., Kesse-Guyot, E., Rambialkowska, E., et al. (2017). Human health implications of organic food and organic agriculture: a comprehensive review. *Enciron. Health* 16, 111. doi: 10.1186/s12940-017-0315-4

Mir, S. A., Shah, M. A., and Mir, M. M. (2017). Microgreens: production, shelf life, and bioactive components. *Crit. Rev. Food Sci. Nutr*. 57, 2730–2736. doi: 10.1080/10408398.2016.1144557

Nunez de Gonzalez, M. T., Osburn, W. N., Hardin, M. D., Longnecker, M., Garg, H. K., Bryan, N. S., and Keeton, J. T. (2015). A survey of nitrate and nitrite concentrations in conventional and organic-labeled raw vegetables at retail. *J. Food Sci.* 80, C942–9. doi: 10.1111/1750-3841.12858

Phillips, K. M., Tarrago-Trani, M. T., McGinty, R. C., Rasor, A. S., Haytowitz, D. B., and Pehrsson, P. R. (2018). Seasonal variability of the vitamin C content of fresh fruits and vegetables in a local retail market. *J. Sci. Food Agric*. 98, 4191–4204. doi: 10.1002/jsfa.8941

Rosen, C. J., and Eliason, R. (2005). *Nutrient Management for Commercial Fruit and Vegetable Crops in Minnesota*. Available online at: https://conservancy.umn.edu/bitstream/handle/11299/197955/nutrient-management-commercial-fruit-vegetable-2005.pdf?sequence=1andisAllowed=y (accessed November 20, 2021).

Schulz, R., and Slavin, J. (2021). Perspective: defining carbohydrate quality for human health and environmental sustainability. *Adv. Nutr*. 12, 1106–1121. doi: 10.1093/advances/nmab050

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*. 5, 1417–1435. doi: 10.3390/nu5041417

Slavin, J., and Lloyd, B. (2012). Health benefits of fruits and vegetables. *Adv. Nutr*. 3, 506–516. doi: 10.3945/an.112.002154

Soetan, K. O., Olaiya, C. O., and Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: a review. *African J. Food Sci.* 4, 200–222. doi: 10.5897/AJFS.9000287

Walker, R. (2009). Nitrates, nitrites and n-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. *Food Addit. Contam.* 7, 717–768. doi: 10.1080/02652039009373938

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Optimization of Protein Quality of Plant-Based Foods Through Digitalized Product Development

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With the increasing availability of plant-based protein products that should serve as alternatives to animal-based protein products, it is necessary to develop not only environmentally friendly but also nutritious foods. Especially the protein content and quality are of concern in these products. The algorithm of NutriOpt was developed using linear programming to support the development of food products with a balanced amino acid profile while considering digestibility. The current version contains a database with 84 plant protein sources from different food groups (legumes, cereals, nuts, seeds) and with different grades of purification (flours, concentrates, isolates) from which NutriOpt can create mixtures with high protein quality while complying with constraints such as protein content, number of ingredients, and weight of the mixture. The program was tested through different case studies based on commercial plant-based drinks. It was possible to obtain formulations with a Protein Digestibility Corrected Amino Acid Score (PDCAAS) over 100 with ingredients and quantities potentially suitable for plant-based analogs. Our model can help to develop the second generation of plant-based product alternatives that can really be used as an alternative on long-term consumption. Further, there is still a great potential of expansion of the program for example to use press cakes or even to model whole menus or diets in the future.

Keywords: plant-based proteins, optimization, protein quality, digital product development, plant-based products, linear optimization

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INTRODUCTION

Environmental degradation and diet-related non-communicable diseases are only a few of the negative outcomes of the high consumption of animal-based products in Western diets (1).

Research has shown that shifting toward a more plant-based dietary pattern can potentially provide major health benefits. For instance, vegans, vegetarians, pescatarians, and semi-vegetarians had a 12 percent lower overall mortality risk than omnivores in the biggest prospective research on vegetarian diets (2). Regarding protein intakes specifically, a prospective cohort analysis indicated that the consumption of plant-based protein instead of animal-based protein was linked to a significant reduction in overall mortality (3).

On the other hand, environmental health also benefits. Because of the non-efficient transformation from plant to animal resources, plant-based foods have generally lower environmental impacts per gram or calorie than animal source foods (4). The high amounts of greenhouse gas (GHG) associated with meat production, as well as the heavy use of water required to raise meat for human consumption, have been found to significantly increase an individual's carbon footprint (5). Concerning vegetable alternatives, several studies show that they produce less GHG emissions and other environmental effect categories like eutrophication and acidification compared to animal-based products (6, 7).

In line with this, diverse types of plant protein ingredients are becoming more and more available, which makes it necessary to investigate and propose methods to develop foods that fulfill nutritional and environmental criteria (8) to support the much-needed decrease in consumption of animal-based sources.

Nevertheless, the plant-based products on the market often have lower protein content and especially lower protein quality. Studies suggest that healthy adults following an entirely plant-based diet living in Western countries with good food accessibility are generally not at risk of protein or amino acid deficiency (9). Yet, other population groups can be prone to deficiency in those nutrients, especially when they replace one-to-one products of animal origin with those based on plants. For instance, plant-based beverages that should replace milk deliver fewer essential amino acids (10). Especially for long-term consumption, this might be crucial. A study demonstrated that children who consumed three cups of non-cow milk - including plant-based beverages- were significantly smaller at the age of three compared to those who drank the same amount of cow milk per day (11). Another study concluded that the primary consumption of the current generation of plant-based beverages during childhood is associated with specific types of illness. For instance, rice beverages were linked to protein malnutrition and almond-based beverages to metabolic alkalosis (12). Furthermore, our previous study showed that the protein quality of vegan diets might be of concern when only low-quality protein sources are consumed (13).

Protein quality (PQ) does not only involve amino acid composition, but also bioavailability (14). All proteins provide the nine indispensable amino acids (IAA) that the human body needs for metabolic functions, but the distribution of these compounds in plant proteins is less optimal than the ones coming from animals (9). To produce foods or diets with an optimum amino acid profile and improved protein quality, combining different plant protein sources is needed (15). For instance, a well-known case is the mixture of legumes, which are high in the amino acid lysine but low in cysteine and methionine, plus cereals which are high in cysteine and methionine but low in lysine (16, 17). However, this "formula" is not a rule, and individual amino acid profiles need to be accounted for, as well as its digestibility and grade of processing (18, 19).

Linear programming can be a tool to aid the development of food items with the aforementioned criteria. For instance, to find combinations of protein ingredients with a good protein quality, with the least cost and environmental impact. To this date, few solutions using this approach are available to develop new food products. Brixi (20) created an algorithm to formulate "ready to use food for the treatment of acute malnutrition" by minimizing the cost of the 26 raw materials in the dataset while satisfying the imposed nutritional criteria, crop water footprint, and ensuring a PDCAAS > 95, whereas De Carvalho et al. (21) programmed an algorithm to support the formulation of low-cost porridges nutritionally suitable for 1–2-year-old children living in rural Mozambique, including a constraint for protein quantity (but not quality).

To close that gap, the aim of the present project was to develop a digital tool to support the formulation of plant-based foods with a high protein quality by creating combinations of different plant protein ingredients with customizable constraints such as the weight of the mixture, the number, and type of protein ingredients while ensuring a high to excellent protein quality.

MATERIALS AND METHODS

First, a literature review was conducted to expand and revise the existing database containing protein, amino acid contents and digestibility values. Afterward, the algorithm NutriOpt was generated through linear programming in R. The algorithm was then tested using case studies, and the protein quality of the resulting plant protein combinations was estimated. The feedback and suggestions from those tests served to make modifications for the existing application (**Figure 1**).

Database Creation

A database of 84 ingredients was set up in an Excel spreadsheet. The ingredients were selected based on their relevance and common use in the meat and dairy analog industry. Each ingredient has a corresponding value on:

- Protein content (g/100 g ingredient).
- Digestible Indispensable Amino Acid (DIAA) content (g/100 g ingredient).

$$DIAA = Content\ of\ each\ IAA\ \ (g/100\ g) \times True\ Protein\ Digestibility\ \ (TPD)$$

- Type of ingredient (i.e., raw grain/seed, protein isolate, protein concentrate).

The values of protein and amino acid content were retrieved in its majority from the USDA database, and the values of TPD from different studies (see **Supplementary Material**).

Furthermore, it contained the IAA target values (scoring pattern) given by the FAO for adults (14).

WebApp

The algorithm was written in R (Shiny) using a linear programming approach to generate combinations of ingredients that meet established amino acid targets, protein content, weight, number and type of ingredients, and (optionally) quantity of a mandatory ingredient. The optimization process will start after the selection of one of the objective functions: the optimal solution under the given constraints will be automatically calculated and a report is generated.

The tool has the following two components:

- Excel spreadsheet used as a database.

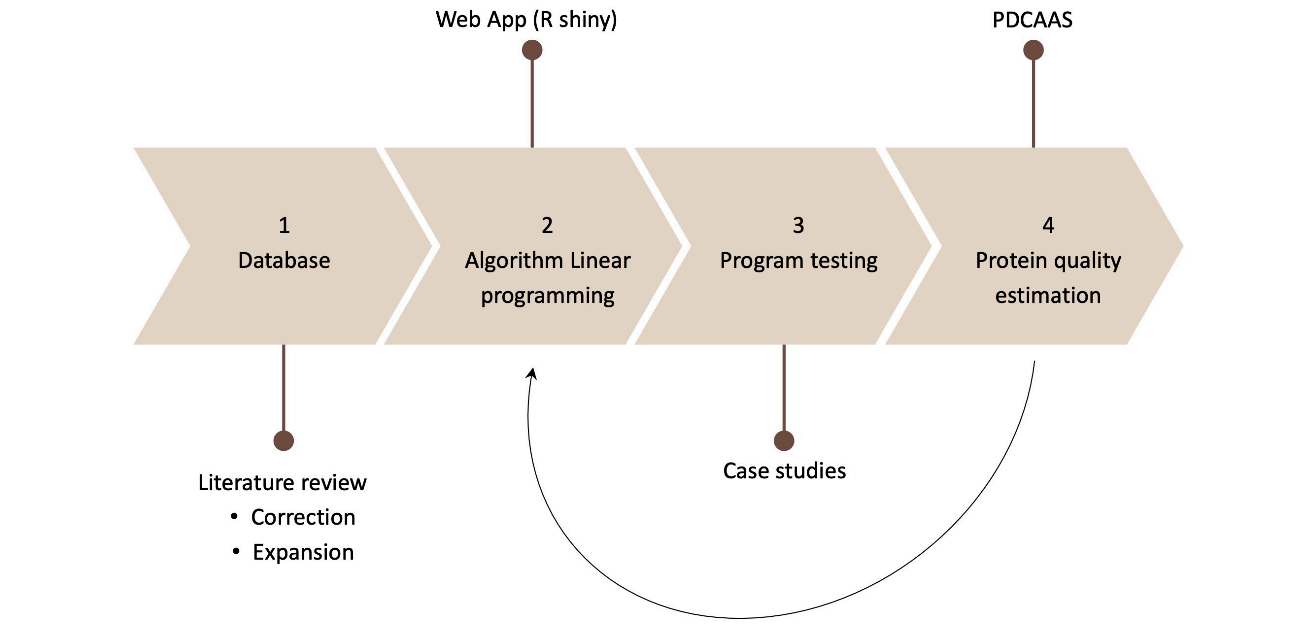

FIGURE 1 | Methodology.

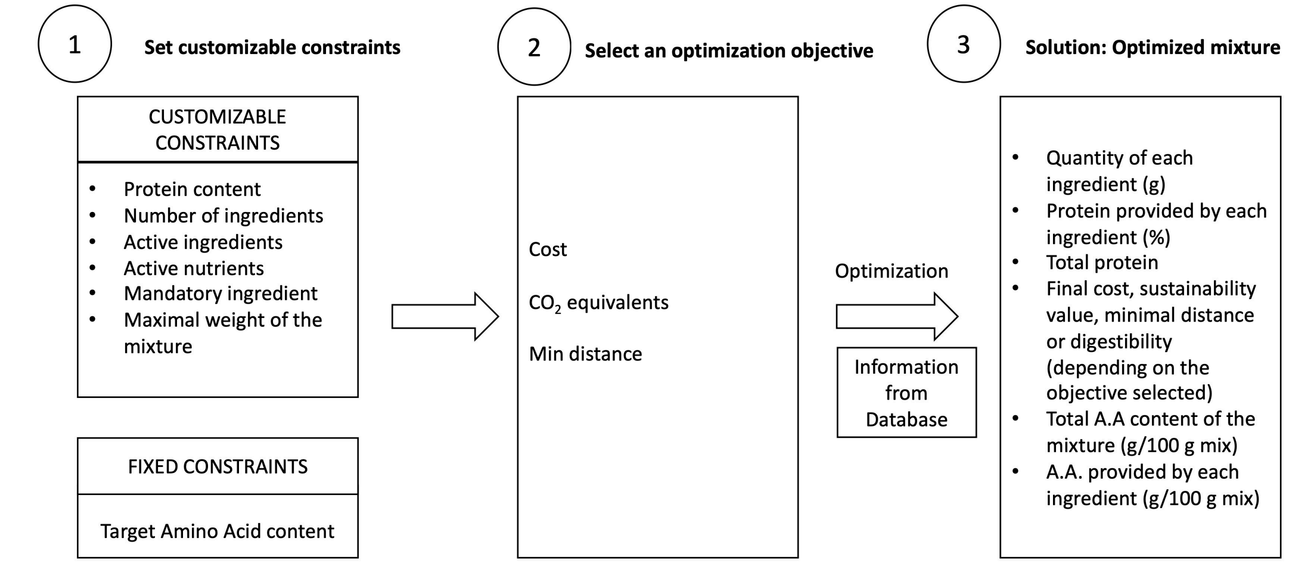

FIGURE 2 | Linear optimization process.

- R Shiny source code file with the algorithm and the settings for the final user.

The WebApp can be launched directly from the source code in the R console (this solution is only available for the project team) or online as WebApp in a web browser (as a test for guests), which is subjected to Password.

The three main steps of the linear optimization process (**Figure 2**) of the WebApp based on the algorithm developed with R Shiny are:

Step 1: The optimization process starts by setting a value for each customizable constraint, namely the parameters that the desired blend will have. When a specific number is not set, the algorithm automatically uses the default value (**Table 1**). There are also fixed constraints, which cannot be changed by the user. They are automatically taken by the algorithm. In this case, the fixed constraints refer to the IAA target values (**Table 2**).

Step 2: An optimization objective is selected: minimal distance (optimized allowed residuum), minimum cost, or minimum CO_2 equivalents. The last two objective functions are fully

TABLE 1 | Customizable constraints of NutriOpt.

	Possible range of selection	Default value
Protein content (g)	0–100	25
Number of ingredients	1–84	5
Activation of ingredients	None – All	All
Activation of nutrients	None – All	All
Mandatory ingredient ("Must be inside product")	0–4 mandatory ingredients, 0–100% of protein contribution	None
Maximal weight of the mixture (g)	0–300	200

implemented in the algorithm, but the database currently contains only arbitrary values to test the correct functionality in the App, therefore in the case studied and presented here, we limit ourselves to the test only the objective function minimal distance.

Step 3: The algorithm shows the optimal solution (if found) in the dashboard (**Figure 3**).

Case Studies

To test NutriOpt, case studies based on plant-based commercial products were used to demonstrate the potential of a real application for the food industry. The main goal was to obtain a combination of ingredients with high to excellent protein quality (PDCAAS > 75) while maintaining similar characteristics (% protein, % protein sources) to market products.

Protein Quality Optimization of an Oat Drink

Based on a commercial oat drink that contains 0.3 g protein/100 g product and the following ingredients: Water, oats flour 13%, hazelnut paste 2%, salt. The objective was to reformulate the plant-based drink to obtain a product with 3 g of protein (similarly to the protein contribution of cow's milk) that still had oats as the main ingredient but substituting the hazelnut paste by two other protein sources derived either from nuts or from seeds. The maximal weight of the mixture was set to 15 g, similarly to the original product. To maintain oat flour as the main ingredient, a constraint of "mandatory ingredient" was set (**Table 3**).

Protein Quality Optimization of a Yogurt Analog

Based on a commercial yogurt analog whose label declares 0 g protein and the following ingredients: water, oat flour 11%, coconut fat, waxy maize starch, thickener (E 412). The objective was to reformulate the product to obtain a product with high to excellent PQ and 3 g of protein (similarly to the protein contribution of regular plain yogurt) and ingredients used already for yogurt analogs (the ingredient of several brands of yogurt analog were consulted and from then, the active ingredients selected) and maintaining oat flour in the list of ingredients. The maximal weight of the mixture was set to 11 g, as the original product (**Table 3**).

Analysis of Optimized Mixtures

Compliance With Constraints

The characteristics of the optimized blend were assessed to verify the compliance with the constraints set. The following checklist was used.

- The ingredient selected "must be inside" is included in the mixture in the indicated percentage.
- The number of ingredients complies with the selected criteria.
- The weight of the mixture complies with the constraint set.
- The indispensable amino acid target is reached (amino acid score).
- The selected amount of protein is reached.
- The activation and inactivation of products is correct as selected by the user.
- The activation and inactivation of nutrients is correct as selected by the user.

Protein Quality

The protein quality of the optimized mixtures was estimated with the Protein Digestibility Corrected Amino Acid Score method (PDCAAS) described by FAO (14) using the amino acid content and digestibility of the raw materials:

$$PDCAAS = \frac{mg\ of\ limiting\ amino\ acid\ in\ 1\ g\ of\ test\ protein}{mg\ of\ the\ same\ amino\ acid\ in\ 1\ g\ of\ reference\ protein} \times True\ fecal\ digestibility$$

RESULTS

It was possible to automatically consider digestibility without having to add an additional constraint, while ensuring a good to excellent protein quality in all the mixtures produced. There was full compliance with the constraints, and no error messages appeared nor were other errors detected. In all assessments the PDCAAS was over 100, indicating an excellent protein quality regardless of the percentage of protein in the mixture.

Protein Quality Optimization of an Oat Drink

The optimized mixture complied with all the constraints and the result was a combination of oat flour, hemp press cake and pumpkin seed flour (**Table 4**). This combination has a similar amount of oat flour compared to the original product. Regarding protein quality, the PDCAAS was 100 (101 not truncated)

TABLE 2 | Fixed nutritional constraints according to the IAA scoring pattern for adults [Adapted from FAO (14)].

	His (g)	Ile (g)	Leu (g)	Lys (g)	SAA (g)	AAA (g)	Thr (g)	Trp (g)	Val (g)
For 25 g of protein	0.375	0.75	1.475	1.125	0.55	0.95	0.575	0.15	0.975
For 1 g of protein	0.015	0.03	0.059	0.045	0.022	0.038	0.023	0.006	0.039

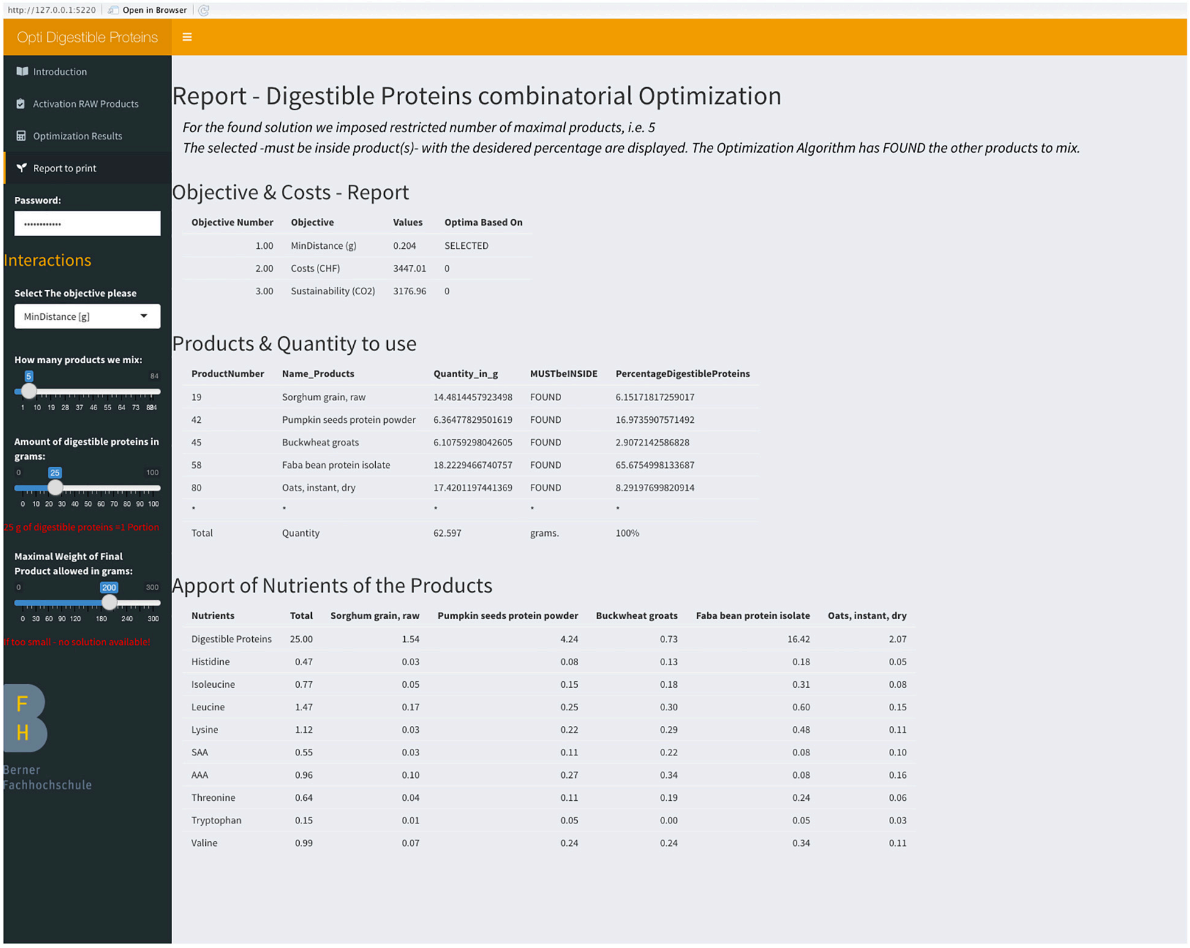

FIGURE 3 | Dashboard of NutriOpt.

(**Figure 4**). Thus, it is classified as high quality. Lysine had the lowest value of the corrected amino acid score, while histidine had the maximal (2.34 times more than the reference pattern).

Compared to the reference product, the optimized formula has a similar quantity of protein ingredients, whereas the protein content was increased from 0.3 to 3 g, respectively. On the other hand, the protein quality also increased: from 60 to 101 (non-truncated value) and from 57 to 94 for adults (>18 years) and older children and adolescents (4–18 years), respectively (**Table 5**).

TABLE 3 | Constraints to optimize two commercial products.

	Oat drink	Yogurt analog
Protein content	3 g	3 g
Number of ingredients	3	2
Maximal weight of the mixture	15 g	11 g
Active ingredients	Oat flour, nuts, and seeds (flours and press cakes)	Oat flour, pea protein isolate, oat protein concentrate, oat protein isolate, chickpea protein isolate
Mandatory ingredients	Oat flour (45% of protein contribution)	NA

Protein Quality Optimization of a Plant-Based Yogurt Analog

For this case study, NutriOpt generated a combination of oat flour and pea protein isolate (**Table 6**), which is in line with the constraints set (only 2 ingredients, 11 g of total weight) and it was

TABLE 4 | Optimized mixture for oat drink.

	Optimized formula	
Ingredient	**Quantity (g)**	**Protein contribution (%)**
Oat flour	10.2 (70%)	45.0%
Hemp press cake	2.6 (18%)	47.1%
Pumpkin seed flour	1.6 (12%)	7.9%
Total	*14.4 (100%)*	*100.0%*

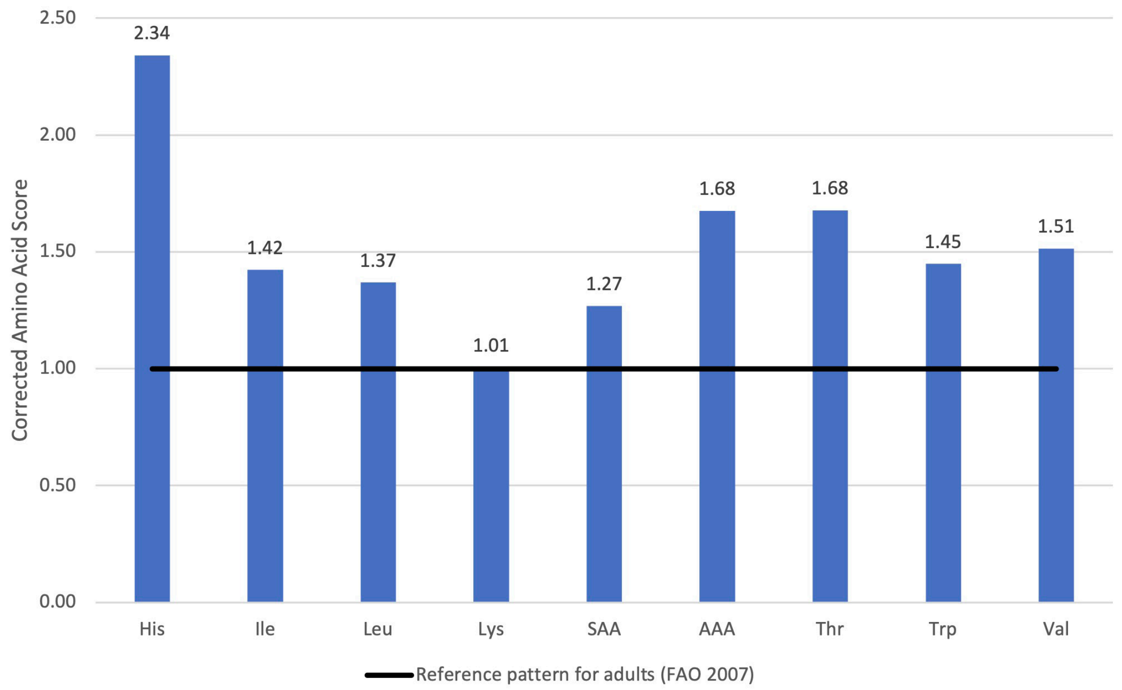

FIGURE 4 | Corrected Amino Acid Score of optimized formula for oat drink.

not necessary to set oat flour as a mandatory ingredient since this was automatically selected for the optimal mixture. Regarding protein quality, the lowest protein corrected amino acid score was 126 for Isoleucine (**Figure 5**). Therefore, as values in PDCAAS are truncated, the final protein quality is reported as 100. All IAA exceed the target set for adults. For the category of older children and adolescents (4–18 years) the estimated PDCAAS is 120, while for preschool children (1–2 years) the PDCAAS is 105 (shown in **Supplementary Material**).

The optimized formula provides 3 g of protein versus the original that declares 0 g. The main protein ingredient in terms of quantity is still oat flour, and by adding 2.4% of pea protein isolate to the formulation, it was possible to increase the protein quality (**Table 7**).

TABLE 5 | Comparison of optimized formula versus original product.

	Original	Optimization
Protein content	0.3 g	3 g
Weight of the mixture	15 g	14.4 g
List of ingredients	Water, oat flour (13%), hazelnut paste (2%), salt	Water, oat flour (10%), hemp protein (2.6%), pumpkin seed protein (1.6%), salt
PDCAAS	60* (>18 years) 57* (4–18 years)	101 (not truncated) (>18 years) 94 (4–18 years)

**Own calculation based on the protein digestibility of raw walnuts and amino acids values from USDA database.*

TABLE 6 | Optimized mixture for yogurt analog.

	Optimized formula	
Ingredient	**Quantity**	**Protein contribution (%)**
Oat flour	8.62 g (78%)	62%
Pea protein isolate	2.38 g (22%)	38%
Total	*11 g (100%)*	*100%*

DISCUSSION

The NutriOpt program can generate blends with a protein quality that is comparable to that of milk protein concentrate (PDCAAS = 125 for the 0.5–3-year-old child) (22). Conversely, the PDCAAS of the raw materials used for some commercial plant-based beverages has been reported to be significantly lower than their animal-based homologs. Namely 68 for quinoa, 63–66 for hemp, 45–60 for oat, 54 for rice, and 30 for almond (23).

In the case studies, the optimized formulas had similar characteristics to the market products regarding proportion and type of protein ingredients, which can facilitate the development process of optimized products. For the yogurt analogs, pea was selected as the complementary protein source. This is a classic example of amino acid complementarity, where a legume, which has a high amount of lysine, but low content of SAA is combined with a cereal that possesses a considerable content of SAA and a low lysine content, resulting in a blend with improved protein quality (16, 17). Nevertheless, the proportion

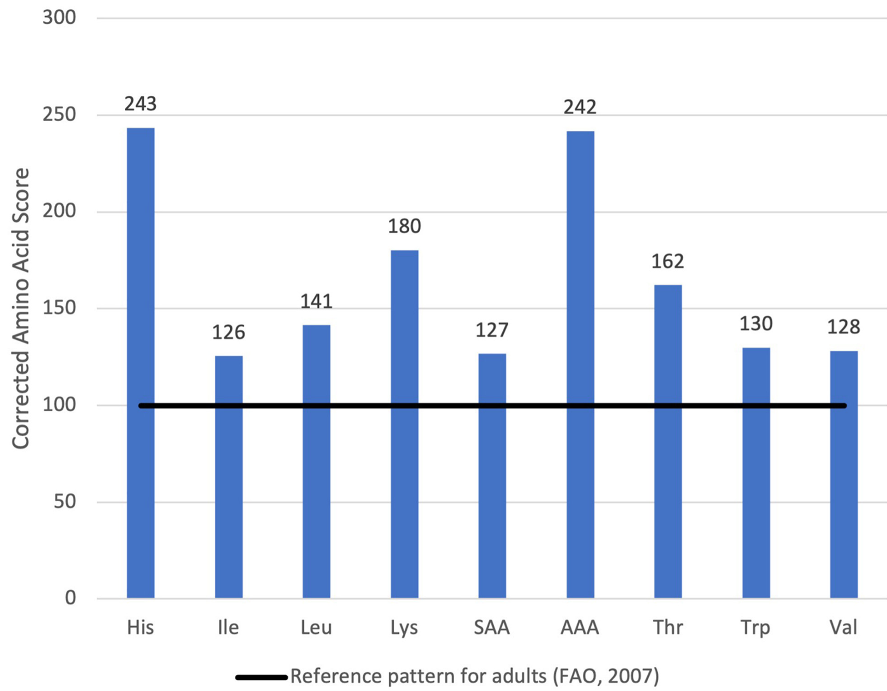

FIGURE 5 | Corrected Amino Acid Score of optimized formula for yogurt analogue.

of the protein sources should not be disregarded because it is not a rule that a legume plus a cereal will have higher PQ scores (24, 25). In both case studies, some amino acids had more than double the amount of the target, but this might be restricted with an additional constraint. Yet, it is important to mention that the addition of a new constraint may decrease the possibility to find a solution. Besides, the constraints need to be logically set. For instance, for obvious reasons, the amount of total protein must be lower than the maximal weight of the mixture. Otherwise, there are no possible solutions.

Limitations inherent to the PDCAAS methodology also limit the interpretation of the results in this study. This approach aims to forecast the utilization of dietary protein to estimate to which degree it can meet the demand for the amino acids necessary for maintenance functions (26). However, it is subject to several limitations. For instance, the erroneous assumption that the digestibility of the crude protein is equal to that of each individual IAA. The absorption rate of these compounds can largely vary between each other, especially in the presence of antinutritional factors (27). Another important shortcoming is the measurement of digestibility along the whole digestive tract (i.e., fecal) which is considered to overestimate the absorption (28). Although the procedure suggests that digestibility values derive from *in vivo* rat analysis, some of the data here were extracted from *in vitro* studies due to availability reasons. Yet, studies indicate that *in vitro* assays can provide an accurate estimate of the True Protein Digestibility (29–31). Despite the disadvantages, PDCAAS has been valuable in practice (26) and has been chosen in this work due to its practicality, acceptability, and the wider availability of data than its counterpart DIAAS.

TABLE 7 | Comparison of optimized yogurt analog formula versus original product.

	Original	Optimization
Protein content	0 g	3 g
Weight of the mixture	11 g	11 g
List of ingredients	Water, oat flour 11%, coconut fat, corn starch, thickener (E 412)	Water, oat flour 8.6%, pea protein 2.4%, coconut fat, corn starch, thickener (E 412)
PDCAAS	0	126 (not truncated) for >18 years 120 (not truncated) for (4–18 years)

Having acknowledged the latter drawbacks, it is important to remark that even though the algorithm is capable to suggest mixtures that reach the digestible amino acid targets and thus a theoretical high PDCAAS, the quality of the results will depend on the quality of the data. Should the database have an erroneous, arbitrary, or missing value, the accuracy of the outcome might be compromised.

Another limitation was the availability of data. To this date, not all the digestibility values and threonine content of the ingredients in the database have been found in the literature: some of them are arbitrarily set or based on assumptions. Therefore, if NutriOpt is to be used in a real setting, these items should be deactivated. It is recommended to update the database information according to the specific ingredient data of the final user.

Moreover, the calculated quality of the protein blends is based on the ingredients as raw materials. However, further processing, the structural organization of the food matrix, and interactions between other ingredients can greatly influence the amino acid content and digestibility of the final product (8, 19, 32, 33). Finally, the sensory and technological performance of the protein combinations provided by NutriOpt is not addressed in the present study and should be subject to test in future research.

A strength of the present project is, however, that the level of refinement in different protein sources was considered. For instance, the availability of one source in different presentations such as raw flour, defatted flour, concentrate, isolate, and cooked form. This individualization provides a more accurate panorama of the amino acid content and bioavailability of the ingredients.

Another advantage is that the database contains a broad range of ingredients that are already being used in the food industry, as well as novel protein sources such as hemp press cake and microalgae protein. This allowed recreating optimized products that are already commercially available. Additionally, most of the data were retrieved from ingredients intended for human food consumption, while other studies in PQ optimization such as that developed by Herreman et al. (24) use datasets from animal nutrition studies, mostly in their raw form.

Given that the algorithm gives a constraint already in the digestible IAA, values not only of good but of excellent protein quality (PDCAAS > 100) are consistently obtained (The values were not truncated for purposes of analysis).

In the future, a wider number of ingredients, organized in categories and groups, can be added. In the view of the circular economy, by-products of oil extraction such as press cakes are a good alternative source of protein. Other optimization objectives can be used such as minimizing cost or CO_2 equivalent, provided that real values are incorporated in the database. Due to time constraints, this work does not include them, but future research can consider them. The estimation of protein quality with the DIAAS method can be implemented when the values of ileal amino acid digestibility are available from new studies.

A version with whole plant-based food (cooked legumes, nuts, cereal-based products) can be set up, prospectively supporting a transition to healthy and sustainable diets, or directed to populations with risk from protein deficiency.

This work is focused on protein, but additional nutritional parameters might be added such as energy, carbohydrates, fat, and fiber, having in consideration that the more constraints present, the smaller number of available solutions.

Assessment of the functionality of the ingredients. It is not clear whether the mixtures are technologically or sensorially appropriate.

Finally, it might be possible to do an automated customization of diets based on specific needs: specific setups for different types of categories of people can be implemented by optimizing the intake of proteins taking into account that the final result is for an athlete, an elderly person, or a person with allergies or food intolerances.

CONCLUSION

There is an increasing number of plant protein ingredients to enable the transition toward more sustainable food production, and such resources must be utilized in a way that provides the consumer with nutritious food alternatives. A constant matter of concern in the transition toward a more plant-based diet is the question of whether the requirements of essential amino acids are being met. A digital tool that provides a combination of ingredients with good and excellent protein quality was developed. This can facilitate the formulation of nutritious products such as, but not limited to dairy/meat analogs, and snacks with a good to excellent protein quality, especially for populations at risk of amino acid deficiency. The novelty of this project is the inclusion of raw materials suitable for the food industry for meat and dairy analogs (protein concentrates, isolates, flours), and the relatively large choice of ingredients in the database: from common ingredients such as soy products to more innovative ones such as microalgae or press cakes. Moreover, the changes in digestibility and amino acid composition are considered due to the inclusion of foodstuffs with different grades of processing, and the digestibility values are taken -when available- from human studies. This program has a great potential for expansion both for food development and for diets. For instance, by amplifying the number and type of ingredients, or by adding data on environmental impact or raw material cost. The question of technological performance remains since the combinations have not been proven in practice, but the customization functions and expert technical insight can serve to deactivate or limit ingredients that, for example, have undesired off-flavors or that provide undesired texture. The algorithm can potentially create solutions with minimum cost or environmental impact, provided adequate data is fed into the database. It is necessary to improve the protein quality of plant-based alternatives of the second generation as long as the consumer uses them really as replacements of the products that originate from animals, especially on long-term. Otherwise, the whole diet must be adapted. Our model can be also used to optimize mixed meals and whole diets in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZR prepared the first version of the manuscript in the frame of her Master thesis, tested the program, and updated the database. KK-B had the idea for the project, conducted the first tests, did the supervision of the nutritional part of the project, and corrected the manuscript. RR was responsible for the digital optimization and the supervision of this part. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.902565/full#supplementary-material

Supplementary Table 1 | Nutritional information of the protein sources and references.

REFERENCES

1. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the anthropocene: the EAT–lancet commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4
2. Orlich MJ, Singh PN, Sabaté J, Jaceldo-Siegl K, Fan J, Knutsen S, et al. Vegetarian dietary patterns and mortality in adventist health study 2. *JAMA Intern Med.* (2013) 173:1230. doi: 10.1001/jamainternmed.2013.6473
3. Song M, Fung TT, Hu FB, Willett WC, Longo VD, Chan AT, et al. Association of animal and plant protein intake with all-cause and cause-specific mortality. *JAMA Intern Med.* (2016) 176:1453. doi: 10.1001/jamainternmed.2016.4182
4. Detzel A, Krüger M, Busch M, Blanco-Gutiérrez I, Varela C, Manners R, et al. Life cycle assessment of animal-based foods and plant-based protein-rich alternatives: an environmental perspective. *J Sci Food Agric.* (2021) 1–13. doi: 10.1002/jsfa.11417
5. Ahnen RT, Jonnalagadda SS, Slavin JL. Role of plant protein in nutrition, wellness, and health. *Nutr Rev.* (2019) 77:735–47. doi: 10.1093/nutrit/nuz028
6. Poore J, Nemecek T. Reducing food's environmental impacts through producers and consumers. *Science.* (2018) 360:987–92. doi: 10.1126/science.aaq0216
7. Smetana S, Mathys A, Knoch A, Heinz V. Meat alternatives: life cycle assessment of most known meat substitutes. *Int J Life Cycle Assess.* (2015) 20:1254–67. doi: 10.1007/s11367-015-0931-6
8. Jiménez-Munoz LM, Tavares GM, Corredig M. Design future foods using plant protein blends for best nutritional and technological functionality. *Trends Food Sci Technol.* (2021) 113:139–50. doi: 10.1016/j.tifs.2021.04.049
9. Mariotti G. Dietary protein and amino acids in vegetarian diets—a review. *Nutrients.* (2019) 11:2661. doi: 10.3390/nu11112661
10. Sousa A, Bolanz KAK. Nutritional implications of an increasing consumption of non-dairy plant-based beverages instead of cow's milk in Switzerland. *J Adv Dairy Res.* (2017) 5:1000197.
11. Morency M-E, Birken CS, Lebovic G, Chen Y, L'Abbé M, Lee GJ, et al. Association between noncow milk beverage consumption and childhood height. *Am J Clin Nutr.* (2017) 106:597–602. doi: 10.3945/ajcn.117.156877
12. Vitoria Miñana I. The nutritional limitations of plant-based beverages in infancy and childhood. *Nutr Hosp.* (2017) 34:1205–14. doi: 10.20960/nh.931
13. Rojas Conzuelo Z, Bez NS, Theobald S, Kopf-Bolanz KA. Protein quality changes of vegan day menus with different plant protein source compositions. *Nutrients.* (2022) 14:1088. doi: 10.3390/nu14051088
14. Joint Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, Weltgesundheitsorganisation, FAO, United Nations University editors. *Protein and Amino Acid Requirements in Human Nutrition: Report of a Joint WHO/FAO/UNU Expert Consultation*. Geneva: WHO (2007). p. 265.
15. Day L. Proteins from land plants – potential resources for human nutrition and food security. *Trends Food Sci Technol.* (2013) 32:25–42. doi: 10.1016/j.tifs.2013.05.005
16. Monnet A-F, Laleg K, Michon C, Micard V. Legume enriched cereal products: a generic approach derived from material science to predict their structuring by the process and their final properties. *Trends Food Sci Technol.* (2019) 86:131–43. doi: 10.1016/j.tifs.2019.02.027
17. Stone AK, Karalash A, Tyler RT, Warkentin TD, Nickerson MT. Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Res Int.* (2015) 76:31–8. doi: 10.1016/j.foodres.2014.11.017
18. Woolf PJ, Fu LL, Basu A. vProtein: identifying optimal amino acid complements from plant-based foods. *PLoS One.* (2011) 6:e18836. doi: 10.1371/journal.pone.0018836
19. Sá AGA, Moreno YMF, Carciofi BAM. Food processing for the improvement of plant proteins digestibility. *Crit Rev Food Sci Nutr.* (2020) 60:3367–86. doi: 10.1080/10408398.2019.1688249
20. Brixi G. Innovative optimization of ready to use food for treatment of acute malnutrition. *Matern Child Nutr.* (2018) 14:e12599. doi: 10.1111/mcn.12599
21. De Carvalho IST, Granfeldt Y, Dejmek P, Håkansson A. From diets to foods: using linear programming to formulate a nutritious, minimum-cost porridge mix for children aged 1 to 2 years. *Food Nutr Bull.* (2015) 36:75–85. doi: 10.1177/156482651503600107
22. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. *J Nutr.* (2015) 145:372–9. doi: 10.3945/jn.114.195438
23. Mäkinen OE, Wanhalinna V, Zannini E, Arendt EK. Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products. *Crit Rev Food Sci Nutr.* (2016) 56:339–49. doi: 10.1080/10408398.2012.761950
24. Herreman L, Nommensen P, Pennings B, Laus MC. Comprehensive overview of the quality of plant – and animal-sourced proteins based on the digestible indispensable amino acid score. *Food Sci Nutr.* (2020) 8:5379–91. doi: 10.1002/fsn3.1809
25. Suri DJ, Tano-Debrah K, Ghosh SA. Optimization of the nutrient content and protein quality of cereal—legume blends for use as complementary foods in Ghana. *Food Nutr Bull.* (2014) 35:372–81. doi: 10.1177/156482651403500309
26. Food and Agriculture Organization of the United Nations editor. *Dietary Protein Quality Evaluation in Human Nutrition: Report of an FAO Expert Consultation, 31 March-2 April, 2011, Auckland, New Zealand*. Rome: Food and Agriculture Organization of the United Nations (2013). p. 66.
27. Capuano E, Oliviero T, Fogliano V, Pellegrini N. Role of the food matrix and digestion on calculation of the actual energy content of food. *Nutr Rev.* (2018) 76:274–89. doi: 10.1093/nutrit/nux072
28. Gilani G, Sepehr E. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. *J Nutr.* (2003) 133:220–5. doi: 10.1093/JN/133.1.220

29. Sauer WC, Ozimek L. Digestibility of amino acids in swine: results and their practical applications. A review. *Livest Prod Sci.* (1986) 15:367–88. doi: 10.1016/0301-6226(86)90076-X
30. Hsu HW, Vavak DL, Satterlee LD, Miller GA. A multienzyme technique for estimating protein digestibility. *J Food Sci.* (1977) 42:1269–73. doi: 10.1111/j.1365-2621.1977.tb14476.x
31. Satterlee LD, Marshall HF, Tennyson JM. Measuring protein quality. *J Am Oil Chem Soc.* (1979) 56:103. doi: 10.1007/BF02671431
32. Bodwell CE, Satterlee LD, Hackler LR. Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymic digestion methods. *Am J Clin Nutr.* (1980) 33:677–86. doi: 10.1093/ajcn/33.3.677
33. Bailey HM, Mathai JK, Berg EP, Stein HH. Pork products have digestible indispensable amino acid scores (DIAAS) that are greater than 100 when determined in pigs, but processing does not always increase DIAAS. *J Nutr.* (2020) 150:475–82. doi: 10.1093/jn/nxz284

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Determinants of Exclusive Breastfeeding and Mixed Feeding Among Mothers of Infants in Dubai and Sharjah, United Arab Emirates

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Background: Breastfeeding (BF) is considered the ultimate method of infant feeding for at least the first 6 months of life. Exclusive breastfeeding (EBF) is one of the most effective interventions to improve child survival. The main objective of this study was to assess the prevalence and duration of exclusive breastfeeding and the associated factors among women in Dubai and Sharjah, UAE.

Methods: A cross-sectional study was conducted in four hospitals and four healthcare centers in Dubai and Sharjah between September 2017 and December 2017. Hospitals and centers are governmental and provide maternal and child health services. A convenience sample of 858 Arab and Emirati mothers with children under the age of 2 years participated in the study. Face-to-face interviews were conducted by using structured questionnaires. The study was approved by the University Ethical Committee and the UAE Ministry of Health before data collection. Descriptive statistics were computed to describe all the questionnaire items. The chi-square test was used to compare the study's categorical variables. A binary logistic regression analysis was used to predict the relationship between BF and its associated factors. Statistical tests with P-values < 0.05 were considered statistically significant.

Results: The mean age of the participating mothers was 30.6 (SD 5.5) years. Results showed that the prevalence of exclusive breastfeeding among the study participants was 24.4% (31.1% in Sharjah and 22% in Dubai; $P = 0.003$). The binary logistic regression reported that mother's and father's education, skin-to-skin period, number of children, mothers' health, and place of living were significantly associated with exclusive breastfeeding ($P < 0.05$). The results reported a significant association between EB and duration of breastfeeding (OR = 6.9, $P = 0.002$), husband education (OR = 2.1, $P = 0.015$), mother education (OR = 1.3, $P = 0.027$), number of children (OR = 7.9, $P = 0.045$), having any health problem (OR = 1.2, $P = 0.045$), and living place (OR = 1.4, $P = 0.033$), and a non-significant positive effect of family size and family income. Furthermore, the result reported a significant association between

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mixed breastfeeding and duration of breastfeeding (OR = 0.1, $P = 0.000$), skin-to-skin period (OR = 0.3, $P = 0.002$), underweight (OR = 4.7, $P = 0.034$), last infant's sex (OR = 1.6, $P = 0.010$), having maid at home (OR = 2.1, $P = 0.000$), number of children (OR = 0.2, $P = 0.013$), and living place (OR =1.1, $P = 0.014$), and a non-significant association with family size and family income.

Conclusions: Therefore, a health promotion program for exclusive breastfeeding during antenatal health visits, together with initiating health policies in maternal hospitals to encourage the initiation of breastfeeding during the first hour of birth and the introduction of skin-to-skin contact during the first 5 min of birth are highly recommended.

Keywords: exclusive breastfeeding, mixed feeding, practices, Sharjah, Dubai, UAE, child under two years

INTRODUCTION

Appropriate feeding practices during infancy and early childhood are essential to meet children's nutritional requirements and to maintain healthy growth and development (1). Substantial evidence supports breastfeeding as the best method for feeding infants and young children, providing them with optimal health and development (2). Accordingly, the World Health Organization (WHO) and the United Nations Children's Funds (UNICEF) recommend that breastfeeding should be initiated early within 1 h of birth, and to continue exclusive breastfeeding with no other foods or liquids for the first 6 months of life (3). This is followed by the introduction of complementary feeding and continued breastfeeding until at least 24 months of age (4).

The benefits of breastfeeding (BF) have been well documented, with solid evidence supporting its impact on reducing the prevalence of both mild and moderate malnutrition as well as childhood diseases (5). Long-term benefits have also been established for BF in terms of the prevention of diseases such as obesity, heart disease, diabetes, and asthma (6–9). However, the WHO and UNICEF have pointed out that the benefits of BF would be achieved when mothers breastfeed their babies for the first 6 months exclusively, i.e., only breast milk (3). Infants who receive any BF would benefit from the nutrients in breast milk and other advantages of BF, such as bonding, cognitive development, and enhancement of the immune system (2). The protective effect from obesity and other childhood diseases on infants and young children fed breast milk might be enhanced through its effect on infant microbiota colonization and development (10, 11).

Breastfeeding directly affects the infant's gut microbiota by exposure to the milk microbiota and indirectly *via* maternal milk factors that affect bacterial growth and metabolism, such as human milk oligosaccharides, secretory IgA, and antimicrobial factors. The potential of breast milk is important in protecting infants from asthma and allergies (12). Among the important core stone benefits of breastfeeding is that it improves child survival in the face of highly infectious diseases like COVID-19 (13). The WHO recommends that breastfeeding should not be discontinued in cases of suspected or being confirmed COVID-19 (13). Thus, the benefit of breastfeeding can overcome the risk of catching the infection as infants acquire passive IgA immunity. This outweighs the potential COVID-19 risks (14).

Despite considerable efforts to promote breastfeeding practices, the Gulf region is still behind when it comes to the goals set by the WHO (15). A study examining breastfeeding practices in the Middle East revealed that a large number of mothers supplemented breastfeeding with other forms of feeding at an early age (16, 17). Since infant nutrition and health are interrelated, the effects of breastfeeding and maternal nutrition on each of these outcomes should be addressed. Diet is an important environmental factor that may influence the health outcomes of breastfeeding mothers and infants. The maternal diet may affect the formation, composition, or secretion of milk. Studies have shown that unhealthy diets and food allergies play a role in the development of asthma in the Gulf countries (18). This finding is of great significance, considering the high prevalence of asthma among children and adults in Gulf countries, to the extent of becoming a significant public health concern.

Similarly, researchers from different countries in Europe have found that breastfeeding practices do not meet the WHO and UNICEF recommendations (19). They pointed out that exclusive breastfeeding practices in different countries in Europe do not meet the 2025 World Health Assembly's Global Target for Nutrition to increase the rate to at least 50% (20). As for the UAE, mixed feeding, complimentary food, and fluid additions have been introduced in the first month of life in the UAE (21). Several factors negatively affect breastfeeding practices in different Gulf countries, such as maternal age, level of education, mothers' perception of insufficient milk production, problems associated with the breast such as nipple problems, mode of delivery (cesarean section), and hospital practices such as non-rooming-in (22, 23). In these studies, a high educational level was more strongly associated with lower BF initiation and exclusive breastfeeding rates. In addition, hospital practices played an important role in breastfeeding outcomes, where vaginal birth and rooming enhanced breastfeeding initiation and extended the breastfeeding duration. In addition, mothers' perception of insufficient milk production and problems associated with the breast, such as nipple problems, have been reported to reduce the rate and duration of exclusive BF. The factors contributing to the continuation of breastfeeding and mixed feeding vary from country to country (24–28). One study in Malawi found that ethnicity of the mother, younger age of the mother, female infant, and high number of children were significantly associated with EBF practices (29). While a study among Cambodian mothers found that those with middle wealth were less likely to go for EBF compared to low wealth mothers (30). Another study among Irish mothers found that maternal age, short maternity leave, mothers from Irish nationality, non-tertiary education, and neonates with intensive-care unit admission were more likely not to adhere to EBF compared to others (31). While concerning Irish primigravida mothers' non-adherence to EBF, the study found that mothers' higher body mass index, unemployment, gestational diabetes, low-birth-weight antenatal steroids, and hypernatremia were all highly associated factors (32). Whereas, reasons for the discontinuation of breastfeeding might include maternal age, educational background, socioeconomic status, postpartum depression, maternal confidence, maternal obesity, and being overweight (33). On the other hand, factors associated with a higher breastfeeding rate and longer duration include increased maternal age, low educational levels, rural residence, low income, multiparity, and avoiding contraceptives (34).

To maintain breastfeeding as the best feeding method that supports infants and young children's health, the WHO has set a global goal to increase the rate of exclusive breastfeeding to at least 50% by 2025 (35). The Ministry of Health in the UAE has made extensive efforts in collaboration with health authorities in all emirates to develop plans and strategies that would help achieve this goal by increasing the rate of EBF (36). As part of these efforts, the UAE has embraced various policy initiatives, including the Baby-Friendly Hospital Initiative (BFHI), the Global Strategy for Infant and Young Child Feeding, and the implementation of the International Code of Marketing of Breast Milk Substitutes (37). According to the MOH national infant feeding policy implemented throughout the country, infants should be breastfed exclusively until 6 months of age (21, 38).

In addition, the UAE Federal National Council passed a draft clause in the child rights law to make breastfeeding mandatory for the first 2 years of an infant's life (39). To support BF among working mothers, a decree was issued that extended the 60 days of paid maternity leave to 90 days. The experience of the Emirate of Sharjah is peculiar, as the city has been recognized as the Middle East's first baby-friendly city following the successful adoption of the main standards for this rating (40). To assess these efforts, it is important to assess breastfeeding practices and determine the EBF rates. Few studies have been conducted on breastfeeding in the UAE and recent studies have been confined to certain emirates. Most of these studies were cross-sectional and only one national survey was conducted in the year 2000. The results of the national survey revealed that only 34% of the infants were exclusively breastfed for up to 4 months of age (36). A recent study conducted in Abu Dhabi reported a rate of 44.3% (41).

Despite the tremendous efforts to increase breastfeeding worldwide, the rates are suboptimal in many countries, including the UAE. In the UAE, there are gaps in understanding why many mothers have difficulties initiating and maintaining exclusive breastfeeding in the first 6 months of life and instead introduce artificial feeding. Therefore, in light of the limited success in EBF promotion, as evidenced by low EBF rates, there are factors affecting infant feeding and breastfeeding practices. Hence, in the current study, exploring these difficulties and associated factors can be amended through education programs and directing governmental intervention efforts to increase the rate of exclusive breastfeeding and meet the WHO and UNICEF goals. In addition, there are ongoing national efforts and investments in these programs, including the development and updating of policies and strategies. However, regardless of the health authorities' efforts to support and promote breastfeeding, the rate of exclusive breastfeeding in the UAE remains suboptimal. Therefore, this study will help assess the prevalence of exclusive breastfeeding and identify other associated factors that impact the duration of exclusive breastfeeding in infants aged 6–24 months in Dubai and Sharjah. Ultimately, the results can help health providers improve mothers' knowledge about breastfeeding. Furthermore, identifying these factors will shed light on why the breastfeeding rates are still suboptimal. Accordingly, there is very little documentation of EBF in the UAE due to the rapid changes in women's lifestyles and engagement in the workforce particularly in Dubai. The main objective of this study was to assess the prevalence of exclusive breastfeeding and to identify the main contributing factors in infants aged 6–24 months in Dubai and Sharjah to improve the public's knowledge and initiate health policies about breastfeeding.

MATERIALS AND METHODS

Study Design and Settings

A cross-sectional study design was used to collect data from the waiting areas of the largest maternal and child outpatient clinics in four hospitals and four health centers in Sharjah and Dubai, UAE.

Data from a convenience sample of 858 mothers were collected between September 2017 and December 2017. Permission and ethical approval to conduct this study was obtained from the University Ethical Committee and the UAE Ministry of Health. Written informed consent was obtained from mothers who met the criteria for this study and were willing to participate. Participants were informed that their participation in this study was voluntary and that they had the freedom to quit the study at any time.

Population and Sampling

To be included in the study, mothers had to be aged 18 years and above, be either Emiratis or Arabs, be able to provide written consent, and have at least one child aged 6 months to 2 years. Participants were excluded if they were <18 years old or had children aged <6 months or more than 2 years. The proposed sample size was to collect data from at least 800 women in waiting areas (100 women from each clinic/center). A total of 858 women (492 living in Dubai and 366 living in Sharjah) participated in this study.

Data Collection

Data were collected using eight trained interviewer-administered multicomponent questionnaires through a structured face-to-face interview at the selected outpatient clinic waiting rooms in hospitals and healthcare centers in Dubai and Sharjah. The research assistant approached mothers visiting outpatient clinics in the waiting rooms of hospitals and public health centers in Dubai and Sharjah and introduced the study with its objectives and protocol. Eligible and interested subjects read and signed a consent form prior to starting face-to-face interviews. A multicomponent questionnaire was developed based on a literature review of similar studies and was reviewed by a panel of experts in the field of infant feeding (42–44). A valid and reliable questionnaire was used to collect the data.

The structured interview questionnaire was translated into Arabic, then back-translated into English, and pilot tested with 66 mothers from one of the hospitals' outpatient clinics in Dubai (the results from the pilot study were not included in this study and were only used for piloting) to ensure the clarity, simplicity, and logical flow of the questions. The questionnaire was revised according to the pilot study. The final version of the questionnaire consisted of 49 questions and required approximately 10–15 min to complete.

The questionnaire consisted of four main sections: sociodemographic data about the mother and the child (17 items; e.g., maternal age, maternal marriage age, mother and father educational level, place of living, maternal employment, family size, number of children, income, having a maid, infant age, birth weight, etc.); family socioeconomic status (five items); knowledge, attitude, and practice of breastfeeding and complementary feeding (27 items); and the mother's obstetric and general health status section (eight items; e.g., type of delivery, lactation amenorrhea, use of contraception, if she is currently pregnant, sore nipples, maternal health perception, and complications), and breastfeeding practices such as (initiation time of breastfeeding, skin-to-skin care duration, breastfeeding duration, infant feeding type).

Anthropometric Measurements

Infant birth weight and length were obtained from the children's health cards, while the mother's weight (kg) and height (cm) were measured during the visit using a standard protocol using the Seca 220 Telescopic Measuring Rod for Column Scales for height/weight measurements. The BMI (kg/m^2) was calculated by dividing the weight (kg) by the height squared (m). The BMI was determined according to the World Health Organization (WHO) classification (45).

Breastfeeding Outcomes

Early initiation of breastfeeding was defined as the proportion of children who latched the breasts within 1 h of birth. Exclusive breastfeeding was defined as an infant fed only breast milk without any other oral intake, except for medications and vitamins, within the last 24 h. Mixed feeding was defined as the introduction of solid food or formula milk during breastfeeding.

Formula feeding was defined as feeding only formula from birth.

Statistical Analysis

Data were entered, cleaned, and analyzed using the Statistical Package for Social Science (SPSS) software version 24. Descriptive statistics were computed to describe all the questionnaire items including frequencies and percentages. Furthermore, inferential statistical analysis, including the chi-square test, ANOVA test, and binary logistic regression analysis (OR) were used to assess the relationships between BF and its associated factors (infant's age, duration of BF, skin-to-skin period, parental education, last infant's sex, maid at the house, birth weight, number of children, health problems, marriage age of mother, family size, family income, and Emirate states). The significant level was set at $P < 0.05$.

RESULTS

The mean age of the participant mothers was 30.6 (SD, 5.5) years. **Table 1** presents the demographic characteristics of women, as about 44% were from Sharjah and the rest were from the Dubai Emirate. Approximately two-thirds of the women were aged between 20 and 34 years. Most women had more than a high school education and were married at the time of the data collection. Only 29% of the women were working, and about two-thirds of the women reported being in the upper- and middle-income groups.

Figure 1 shows the prevalence rates of different breastfeeding practices: EBF (24.4%), predominant breastfeeding (20.0%), and mixed feeding (57.1%).

Tables 2, **3** present the associations between EBF and mixed feeding (breast milk and formula milk), and the selected sociodemographic characteristics. Significant associations were found between the place of residence, employment, and EBF ($P < 0.05$). Participants living in the Emirate of Sharjah and non-working women had more EBF than those living in Dubai and working women ($P = 0.003$).

The results showed that working women and those who had a maid at home were significantly associated with mixed feeding ($P < 0.001$; **Table 3**).

Table 4 shows the associations between EBF and a mother's obstetric and general health status variables as type of delivery methods; being pregnant; use of contraceptives; complaints of sore nipple; maternal overall health status, and body mass index (BMI) were not significantly associated with EBF ($P =$ 0.796, 0.192, 0.409, 0.364, 0.192), respectively. Only lactational amenorrhea during breastfeeding was significantly associated with EBF ($P < 0.001$).

Regarding mixed feeding and women's overall health status, as shown in **Table 5**, those who used contraceptives were more likely to use mixed feeding than those who did not ($P < 0.010$). In addition, women who had amenorrhea while breastfeeding were more likely not to use mixed feeding than those who did not ($P < 0.000$).

Table 6 shows that women who started breastfeeding soon after delivery in <1 h were significantly associated with EBF ($P < 0.010$). In addition, a longer breastfeeding period was significantly associated with EBF ($P < 0.000$).

The results of the logistic regression in **Table 7** indicate that exclusive breastfeeding and mixed breastfeeding of mothers living in the UAE are affected by many factors. The determinants

TABLE 1 | Sociodemographic characteristics of participating women in both Dubai and Sharjah.

Sociodemographic characteristics		Emirate (*n* = 858)					
		Sharjah		Dubai		Total	
		n	%	*n*	%	*n*	%
Mother education	High school and less	107	26.7	168	34.1	275	30.8
	Higher than high school	294	73.3	325	65.9	619	69.2
Mother occupation	Working	83	20.7	180	36.5	263	29.4
	Not working	318	79.3	313	63.5	631	70.6
Husband education	High school and less	89	22.2	152	30.8	241	27.0
	Higher than high school	312	77.8	341	69.2	653	73.0
Husband's working status	Working fulltime	399	99.5	480	97.4	879	98.3
	Not working	2	0.5	13	2.6	15	1.7
Family income	Middle and lower income	187	57.7	69	15.2	256	32.9
	Upper than middle income	137	42.3	386	84.8	523	67.1
Family size	≤4	214	57.7	237	50.6	451	53.8
	>4	157	42.3	231	49.4	388	46.2
Number of children	<3	248	61.8	290	58.8	538	60.2
	≥3	153	38.2	203	41.2	356	39.8
Marital status	Married	399	99.5	473	95.9	872	97.5
	Divorced or widow	2	0.5	20	4.1	22	2.5
What is your last infant's sex?	Boys	222	55.4	254	51.5	476	53.2
	Girls	179	44.6	239	48.5	418	46.8
Age group	15–19	5	1.3	4	0.8	9	1.0
	20–34	307	77.5	350	74.3	657	75.8
	35–50	84	21.2	117	24.8	201	23.2
Infants age (month)	1–6	68	17.0	52	10.5	120	13.4
	7–12	171	42.6	176	35.7	347	38.8
	13–18	115	28.7	161	32.7	276	30.9
	≥19	47	11.7	104	21.1	151	16.9

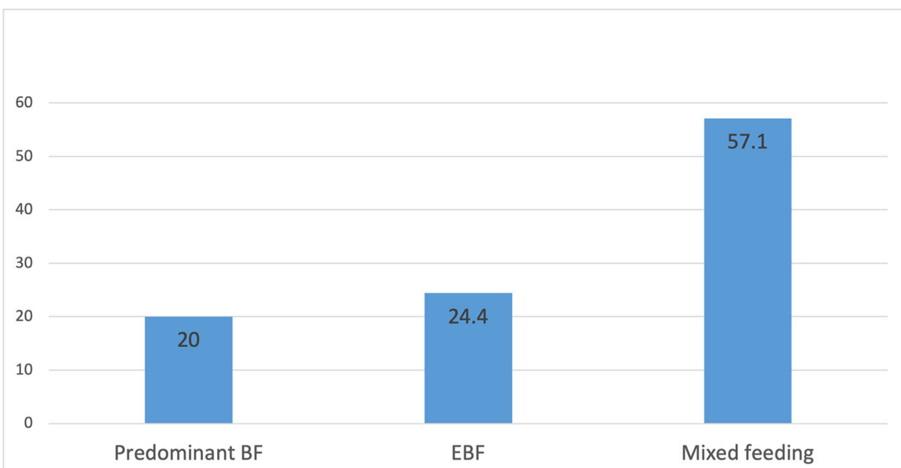

FIGURE 1 | Prevalence breastfeeding practices among women (predominant breastfeeding, exclusive breastfeeding and mixed feeding).

of breastfeeding indicated by "exclusive and mixed formula breastfeeding" among Emirate mothers are in the overall population, before and during the pandemic, is assessed by several variables of which duration of breastfeeding, skin-to-skin period, having made at home, having any health problem, family income, gender, infant age, family size, and family income.

TABLE 2 | Association between EBF and women's sociodemographic characteristics.

Sociodemographic characteristics		Exclusive breastfeeding (*n* = 858)						*P*-Value
		Yes		No		Total		
		n	%	*n*	%	*n*	%	
Emirate	Sharjah	119	54.6	263	42.8	382	45.9	0.003*
	Dubai	99	45.4	351	57.2	450	54.1	
Marriage age of mother	≤20	75	35.0	175	28.6	250	30.3	0.079
	>20	139	65.0	436	71.4	575	69.7	
Marital status	Married	213	97.7	600	97.7	813	97.7	0.991
	Divorced or widow	5	2.3	14	2.3	19	2.3	
Mother education	High school and less	68	31.2	193	31.4	261	31.4	0.948
	Higher than high school	150	68.8	421	68.6	571	68.6	
Mother occupation	Working	41	18.8	199	32.4	240	28.8	0.000*
	Not working	177	81.2	415	67.6	592	71.2	
Husband education	High school and less	53	24.3	182	29.6	235	28.2	0.133
	Higher than high school	165	75.7	432	70.4	597	71.8	
Husband occupation	Working fulltime	217	99.5	603	98.2	820	98.6	0.156
	Not working	1	0.5	11	1.8	12	1.4	

**P-value significant at P = <0.005.*

TABLE 3 | Association between mixed feeding (breast milk and formula milk and women's sociodemographic characteristics.

Women characteristics		Mixed feeding (breast milk + formula milk) (*n* = 858)						*P*-Value
		Yes		No		Total		
		n	%	*n*	%	*n*	%	
Emirate	Sharjah	192	44.1	190	47.9	382	45.9	0.282
	Dubai	243	55.9	207	52.1	450	54.1	
Marriage age of mother	≤20	122	28.2	128	32.7	250	30.3	0.162
	>20	311	71.8	264	67.3	575	69.7	
Marital status	Married	428	98.4	385	97.0	813	97.7	0.173
	Divorced or Widow	7	1.6	12	3.0	19	2.3	
Mother education	High School and Less	128	29.4	133	33.5	261	31.4	0.206
	Higher than High School	307	70.6	264	66.5	571	68.6	
Mother occupation	Working	158	36.3	82	20.7	240	28.8	0.000*
	Not Working	277	63.7	315	79.3	592	71.2	
Having maid at home	Yes	226	69.1	173	42.2	435	57.1	0.000*
	No	117	30.9	210	54.8	372	42.9	
Husband occupation	Working fulltime	428	98.4	392	98.7	820	98.6	0.673
	Not working	7	1.6	5	1.3	12	1.4	

**P-value significant at P = <0.005.*

To explain exclusive breastfeeding, the odds ratio (OR) of women with infants' age >12 months are [0.6, 0.8 (95% C. I: (0.1–2.5), (0.1–4.2)); 1.8, 2.7 (95% C.I: (0.5–6.2), (0.6–11.5))] for EBF and mixed BF, respectively. Predictors (breastfeeding duration, skin-to-skin period, fathers' and mothers' educational levels, number of children, and place of residence) were significant for exclusive breastfeeding. For mixed breastfeeding, the predictors (duration of breastfeeding, skin-to-skin period, infant underweight, infant sex, maid at home, number of children, and living place) were significant with mixed BF variables. The three highest OR values were found in ≥13 months duration of breastfeeding, the number of children, >10 husband education predictors [OR (95% C.I): 7.9 (1–65.2), 6.9 (2–23.8), 2.1 (0.8–5.5)] for the EBF, respectively. While the highest of the three OR values is found in family size >4 members, underweight infants, and infants age ≥19 months [OR (95% C.I): 7.1 (1.8–28.3); 4.7 (0.4–50.2); 2.7 (0.6–11.5)] for the mixed BF variable.

TABLE 4 | Association between EBF and women's obstetric and general health status variables.

Women's overall health status		Exclusive breastfeeding (*n* = 858)						*P*-Value
		Yes		No		Total		
		n	%	*n*	%	*n*	%	
Delivery mode	Normal	154	70.6	428	69.7	582	70.0	0.796
	Cesarean	64	29.4	186	30.3	250	30.0	
Pregnancy status	Yes	27	12.4	57	9.3	84	10.1	0.192
	No	191	87.6	557	90.7	748	89.9	
Do you have any health problems?	Yes	25	11.5	89	14.5	114	13.7	0.264
	No	193	88.5	525	85.5	718	86.3	
Contraceptive use	Yes	52	23.9	164	26.7	216	26.0	0.409
	No	166	76.1	450	73.3	616	74.0	
Sore nipples	Yes	76	34.9	234	38.1	310	37.3	0.364
	No	142	65.1	380	61.9	522	62.7	
lactation amenorrhea	Yes	138	63.3	267	43.5	405	48.7	0.000
	No	80	36.7	347	56.5	427	51.3	
Mother BMI	Underweight (<18.5)	3	1.5	11	1.9	14	1.8	0.078
	Normal (18.5–25)	93	46.5	213	37.2	306	39.6	
	Overweight (26–29)	69	34.5	208	36.3	277	35.8	
	Obese (≥30)	35	17.5	141	24.6	176	22.8	

**P value significant at P = < 0.001.*

TABLE 5 | Association between mixed feeding (breast milk and formula milk) and women's health status.

Women's health characteristics		Mixed feeding (breast milk + formula milk) (*n* = 858)						*P*-Value
		Yes		No		Total		
		n	%	*n*	%	*n*	%	
Type of delivery	Normal	294	67.6	288	72.5	582	70.0	0.119
	Cesarean	141	32.4	109	27.5	250	30.0	
Pregnancy status	Yes	41	9.4	43	10.8	84	10.1	0.509
	No	394	90.6	354	89.2	748	89.9	
Do you have any health problems?	Yes	64	14.7	50	12.6	114	13.7	0.375
	No	371	85.3	347	87.4	718	86.3	
Contraceptive use	Yes	130	29.9	86	21.7	216	26.0	0.007
	No	305	70.1	311	78.3	616	74.0	
Sore nipples	Yes	167	38.4	143	36.0	310	37.3	0.480
	No	268	61.6	254	64.0	522	62.7	
lactational amenorrhea	Yes	165	37.9	240	60.5	405	48.7	0.000
	No	270	62.1	157	39.5	427	51.3	
Mother BMI	Under weight (<18.5)	7	1.7	7	1.9	14	1.8	0.515
	Normal (18.5–25)	160	38.6	146	40.7	306	39.6	
	Over Weight (26–29)	144	34.8	133	37.0	277	35.8	
	Obese (≥30)	103	24.9	73	20.3	176	22.8	

**P value significant at P = <0.005.*

The effects of the mother's education, father's education, skin-to-skin period, number of children, mother's health, and living region reported an increase in the odds ratio. Additionally, they were more likely to breastfeed exclusively. The odds ratio of the breastfeeding duration and skin-to-skin period showed a significant and negative effect on mixed breastfeeding, indicating an association with a decreased odds ratio of mixed breastfeeding. Furthermore, the results reported a significant and positive impact of having a maid at home, family size, and family income, indicating an association with an increased odds ratio of mixed breastfeeding.

TABLE 6 | Association between EBF and different feeding practices.

Women's breastfeeding practices		Exclusive breastfeeding (n = 858)						*P*-Value
		Yes		No		Total		
		n	%	*n*	%	*n*	%	
Breastfeeding during the 1st hour after birth	Yes	174	79.8	429	69.9	603	72.5	0.005
	No	44	20.2	185	30.1	229	27.5	
When did you start breastfeeding?	Directly after delivery (within the first hour)	174	79.8	429	69.9	603	72.5	0.007
	After one hour	35	16.1	113	18.4	148	17.8	
	After 1 day	5	2.3	40	6.5	45	5.4	
	After few days	4	1.8	32	5.2	36	4.3	
Infant still breastfeeding	Yes	124	56.9	211	34.4	335	40.3	0.000
	No	94	43.1	402	65.5	496	59.6	
Breastfeeding duration	≤6months	37	38.9	271	65.9	308	60.9	0.000
	>6months	58	61.1	140	34.1	198	39.1	
Skin to skin contact period	≤5 min	75	43.9	188	42.0	263	42.5	0.661
	6–10 min	31	18.1	96	21.4	127	20.5	
	>10	65	38.0	164	36.6	229	37.0	

**P-value significant at P = < 0.005.*

DISCUSSION

Breastfeeding provides both mothers and infants with great benefits and is highly recommended for all mothers. This study assessed the prevalence of exclusive breastfeeding and the determinant factors influencing exclusive breastfeeding practices among women living in Dubai and Sharjah. The study showed that only 24.4% of women practiced EBF. Despite the WHO recommendations regarding breastfeeding and EBF benefits for both infant growth and reduction in the risk of diseases, women in the UAE are still far from reaching the target WHO goal (1). In comparison, more than half of the women practiced mixed feeding (57.1%) in both Dubai and Sharjah, which is similar to a previous study in the UAE among Emirate women, which reported that only 24% of the participants have exclusively breastfed their infants (21). This indicates that the practices of breastfeeding did not change despite all the national efforts (37).

Our study showed that the main factors associated with women refraining from EBF were being working women and living in Dubai. This was similar to a study conducted in Abu Dhabi, where 60% of the working women stopped breastfeeding (22). In the UAE, the women's labor force increased dramatically between 1990 and 2019 at the rates of 28.9 and 52.39%, respectively (46), and further rose to 57.5 in 2020 according to the World Bank (47), which increased the number of working women in the UAE. Therefore, our results may reflect the barriers faced by working mothers in the UAE, which are deterrents to breastfeeding. This might be because of the number of working hours or duration of maternal leave. In addition to the lack of nurseries in the mother's workplace, making it difficult for working women to breastfeed their infants (22). Previous studies have also reported that maternal employment was negatively associated with exclusive breastfeeding (16, 48–50). However, this study showed that women living in Sharjah were more committed to EBF than those living in Dubai. This may be related to several factors, such as the EBF education and awareness programs in Sharjah (40).

On the other hand, the results showed that women who had a maid at home (70%) were more likely to mixed feed their infants than those who had no maids at home. This might be related to the fact that women stay away for long hours from their infants; it would be much easier for the maid to control the infant's hunger by using formula milk when the mother is away from home. This is consistent with a study conducted in Saudi Arabia on the effect of having a maid on raising children and mothers' attachment. It was found that more than half of the maids were responsible for both household cleaning and nourishing the infants, and were mainly using bottle feeding because it is more convenient and fast, especially when the mother is working and away from home (51).

Our study showed that mothers who did not experience lactation amenorrhea and used contraceptives were more prone to mixed feeding. The relationship between menstruation (ovulation) and breastfeeding has a positive relationship; in that, studies have shown that the more frequent breastfeeding and the duration of breastfeeding, the longer extended period of menstrual cycle stopping among women. Therefore, it is expected that women who do not breastfeed more frequently will have their ovulation sooner than those who adhere to the frequency of breastfeeding. This was found in earlier studies in Bangladesh that resumed the menstrual cycle and mixed feeding, among which the study by Radwan (52).

Early skin-to-skin contact with the newborn after delivery was found in our study to predict EBF practices, similar to that found by Moor et al. (53), as this contact would create an intimate relationship and interaction between and build feelings of warmth, care, and connection. Skin-to-skin contact also enhances the release of oxytocin hormone,

TABLE 7 | Odds ratio between EBF and mixed feeding together with some variables.

Variables		Exclusive breastfeeding				Mixed breastfeeding			
		OR	CI 95%		*P*-Value	OR	CI 95%		*P*-Value
Infants age (month)	1–6	1.0				1.0			
	7–12	1.1	0.3	4.5	0.361	1.2	0.4	3.8	0.297
	13–18	0.6	0.1	2.5	0.397	1.8	0.5	6.2	0.325
	≥19	0.8	0.1	4.2	0.898	2.7	0.6	11.5	0.755
Duration of breastfeeding	0–6 months	1.0				1			
	7–12 months	2.0	0.8	4.7	0.051	0.2	0.1	0.5	0.000
	≥13 months	6.9	2.0	23.8	0.002	0.1	0.0	0.2	0.000
Skin to skin period	≤5 min	1.0				1			
	6–10 min	0.8	0.3	2.3	0.066	0.7	0.3	1.7	0.234
	>10	2.0	0.9	4.5	0.042	0.3	0.2	0.6	0.002
Underweight	No	1.0				1			
	Yes	0.7	0.1	7.2	0.722	4.7	0.4	50.2	0.034
Husband education	High school and less	1.0				1			
	Higher than high school	2.1	0.8	5.5	0.015	0.6	0.3	1.2	0.241
Mother education	High school and less	1.0				1			
	Higher than high school	1.3	0.5	3.5	0.027	1.4	0.6	3.1	0.180
What is your last infant's sex?	Girls	1.0				1			
	Boys	0.7	0.3	1.4	0.180	1.6	0.9	2.9	0.010
Do you have a maid at home?	No	1.0				1			
	Yes	0.8	0.4	1.6	0.072	2.1	1.1	4.0	0.000
Birth weight	Normal birth weight	1.0				1			
	Low birth weight	1.1	0.3	4.8	0.920	0.8	0.2	3.0	0.680
Number of children	<3	1.0				1			
	≥3	7.9	1.0	65.2	0.045	0.2	0.0	0.6	0.013
Do you have any health problem?	No	1.0				1			
	Yes	1.2	0.4	3.2	0.045	0.9	0.4	2.1	0.065
Marriage age of mother	≤20	1.0				1			
	>20	0.7	0.3	1.6	0.320	1.3	0.6	2.5	0.255
Family size	≤4	1.0				1			
	>4	0.1	0.0	1.1	0.271	7.1	1.8	28.3	0.926
Family income	Middle and lower income	1.0				1			
	Upper than Middle income	1.0	0.4	2.5	0.468	2.5	1.1	5.7	0.755
Emirate	Dubai	1.0				1			
	Sharjah	1.4	0.6	3.5	0.033	2.5	1.1	5.8	0.014

**P-value significant at P = <0.050.*

which is beneficial for controlling postpartum hemorrhage (53). It was also found in one study by Conde-Agudelo et al. (54) that the Kangaroo strategy of skin-to-skin contact together with exclusive breastfeeding would decrease the infant mortality rate.

The study showed that breastfeeding in the first hour after delivery is highly associated with EBF, in that the production of milk will be initiated, and women would feel more satisfied with their infant needs. While infants might feel attached to breastfeeding and be more connected to their mothers, eventually leading to a longer breastfeeding period and an increased EBF commitment. Early initiation of breastfeeding is highly recommended by the WHO and UNICEF (4). Early initiation was also found to be significantly associated with EBF in our study; the earlier it started, the more committed. Many studies support the importance of initiating breastfeeding and the relationship with EBF (22, 55). In this study, a high income was also found to be strongly associated with mixed feeding. Similarly, in a previous study in UAE, high income was associated with the cessation of breastfeeding (22). High income was found to be associated with cessation of breastfeeding in several other studies worldwide (56, 57).

The study also showed that women who breastfed their infants for more than 6 months and those who currently breastfed their infants were significantly more likely to undergo EBF than the others. This finding was consistent with the results of a study conducted in Cyprus (58). This could be related to the fact that women who feel committed to breastfeeding their infants will be more likely to dedicate themselves to EBF at an early stage; on the other hand, women who choose not to feed for more than 6 months would be more likely not highly dedicated and committed to EBF and prone to mixed feeding.

Among the predictors of mixed feeding, our study showed infant's underweight, mother's education, infant gender, having a maid at home, mother's age at marriage, family size, family income, and living place are significant. Infant underweight is culturally associated with insufficient milk production in the Arab and Gulf countries, or the milk is not very nutritious for the baby; therefore, many mothers tend to go for mixed feeding and eventually, after some time, cessation of breast milk. This is consistent with many studies and has been discussed among several other cultures and Arab cultures (21, 38, 59, 60). It was specifically reported in an early study that women in the UAE initiated mixed feeding as early as the first month of infancy for the same reason (21).

Interestingly, our study did not show a significant association between maternal health problems, method of delivery, and maternal BMI with EBF, although it was previously found to be among the determinants of EBF in the Gulf countries (22, 23, 41). Regarding the mode of delivery, although it showed no significant association with EBF, more than half of the women who delivered *via* cesarean section reported mixed feeding, which is similar to previous studies because of operation pain and discomfort (22, 41, 60). Regarding women's BMI and EBF, it was found previously that maternal obesity was considered a risk factor for initiating breastfeeding among women in developing countries (61). Since the physiological and psychological determinants among obese women prevent them from initiating breastfeeding, neither sustain the practice for a longer period, looking to the fact that prolactin production is lower, big breasts with large areola and inverted nipples make breastfeeding difficult (62). Our study showed that approximately 51% of the obese and overweight participants fed their children mixed feeding, compared to 22% who practiced EBF. This could tell you that despite the difficulties that weight can endorse, those who are willing to feed their children mother's milk were more committed as in the Arab culture the obese women have more nutritional milk than underweight women, and therefore family might support breastfeeding.

Some limitations of this study should be considered when interpreting its results. First, the data in this study represent two out of the seven UAE emirates. Although Dubai and Sharjah are two of the most densely populated emirates, geographical differences may exist when all seven emirates are considered. Another limitation was the small number of participants who met the study criteria for selection and missing data for some variables. Therefore, the results are not generalizable to the entire UAE, and additional research to cover all the seven emirates is needed. Another limitation of this study was the recall bias. Recall bias was common among the participants who were interviewed about past events.

CONCLUSION

This study has highlighted several important findings. Mothers who were not working and those living in Sharjah had a higher prevalence of EBF. Other factors associated with EBF were early skin-to-skin contact and breastfeeding during the first-hour post-delivery. Further research to cover all the other seven emirates and determinant factors for EBF is recommended to encourage breastfeeding-supportive working environment policies, a health promotion program for exclusive breastfeeding during antenatal health visits, together with initiating health policies in maternal hospitals to encourage the initiation of breastfeeding during the first hour of birth and the introduction of skin-to-skin contact during the first 5 min of birth is highly recommended. Future studies regarding the effect of EBF on infant growth and development in the UAE are highly encouraged.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Both Zayed University Ethical Committee and the UAE Ministry of Health Ethical Committee approved this study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HA designed the study and HA and HR recruited the participants and supervised the data collection. HA and RQ analyzed the data. HA, EA, and ZT wrote the manuscript. HA designed the study and manuscript writing. HR reviewed the manuscript. All contributed authors of this original manuscript authorized the final version of the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

1. Heatley ML, Watson B, Gallois C, Miller YD. Women's perceptions of communication in pregnancy and childbirth: influences on participation and satisfaction with care. *J Health Commun.* (2015) 20:827–34. doi: 10.1080/10810730.2015.1018587
2. Dieterich CM, Felice JP, O'Sullivan E, Rasmussen KM. Breastfeeding and health outcomes for the mother-infant dyad. *Pediatr Clin North Am.* (2013) 60:31–48. doi: 10.1016/j.pcl.2012.09.010
3. Saadeh MR. A new global strategy for infant and young child feeding. *Forum Nutr.* (2003) 56:236–8.
4. WHO. World Health Organization. *Guiding Principles for Complementary Feeding of the Breastfed Child*. Washington DC: WHO, Protection DoHPa, Program FaN (2003). Available online at: https://apps.who.int/nutrition/topics/complementary_feeding/en/index.html
5. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *Lancet.* (2013) 382:452–77. doi: 10.1016/S0140-6736(13)60996-4
6. Arenz S, Ruckerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity–a systematic review. *Int J Obes Relat Metab Disord.* (2004) 28:1247–56. doi: 10.1038/sj.ijo.0802758
7. Cope MB, Allison DB. Critical review of the World Health Organization's (WHO) 2007 report on 'evidence of the long-term effects of breastfeeding: systematic reviews and meta-analysis' with respect to obesity. *Obes Rev.* (2008) 9:594–605. doi: 10.1111/j.1467-789X.2008.00504.x
8. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, et al. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid Rep Technol Assess*. (2007) 153:1–186.
9. Lodge CJ, Tan DJ, Lau MX, Dai X, Tham R, Lowe AJ, et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *APA Acta Paediatrica.* (2015) 104:38–53. doi: 10.1111/apa.13132
10. Koleva PT, Bridgman SL. Kozyrskyj AL. The infant gut microbiome: evidence for obesity risk and dietary intervention. Nutrients. (2015) 7:2237–60. doi: 10.3390/nu7042237
11. Alsharairi NA. The infant gut microbiota and risk of asthma: the effect of maternal nutrition during pregnancy and lactation. *Microorganisms.* (2020) 8:1119. doi: 10.3390/microorganisms8081119
12. van den Elsen LWJ, Garssen J, Burcelin R, Verhasselt V. Shaping the gut microbiota by breastfeeding: the gateway to allergy prevention? *Front Pediatr.* (2019) 7:47. doi: 10.3389/fped.2019.00047
13. WHO. *Breastfeeding and COVID-19, Scientific Brief*. Geneva: WHO (2020).
14. WHO. World Health Organization. *Clinical Management of COVID-19: Interim Guidance, 27 May 2020*. Geneva: World Health Organization (2020) 2020. Contract No.: WHO/2019-nCoV/clinical/2020.5. doi: 10.15557/PiMR.2020.0004
15. WHO. World Health Organization. *World Health Statistics.* Geneva: WHO (2015). Available online at: https://apps.who.int/iris/handle/10665/170250
16. Gardner H, Green K, Gardner A. Infant feeding practices of Emirati women in the rapidly developing city of Abu Dhabi, United Arab Emirates. *Int J Environ Res Public Health.* (2015) 12:10923–40. doi: 10.3390/ijerph120910923
17. Hwalla N, Al Dhaheri AS, Radwan H, Alfawaz HA, Fouda MA, Al-Daghri NM, et al. The prevalence of micronutrient deficiencies and inadequacies in the Middle East and approaches to interventions. *Nutrients*. (2017) 9:229. doi: 10.3390/nu9030229
18. Alsharairi NA. Diet and food allergy as risk factors for asthma in the Arabian Gulf region: current evidence and future research needs. *Int J Environ Res Public Health*. (2019) 16:3852. doi: 10.3390/ijerph16203852
19. Zakarija-Grković I, Cattaneo A, Bettinelli ME, Pilato C, Vassallo C, Borg Buontempo M, et al. Are our babies off to a healthy start? The state of implementation of the Global strategy for infant and young child feeding in Europe. *Int Breastfeed J*. (2020) 15:51. doi: 10.1186/s13006-020-00282-z
20. World Health Organization. *Global Nutrition Monitoring Framework: operational guidance for tracking progress in meeting targets for 2025.* Geneva: World Health Organization (2017). Available online at: https://creativecommons.org/Licenses/by-nc-sa/3.0/igo
21. Radwan H. Patterns and determinants of breastfeeding and complementary feeding practices of Emirati Mothers in the United Arab Emirates. *BMC Public Health.* (2013) 13:171. doi: 10.1186/1471-2458-13-171
22. Taha Z, Hassan AA, Wikkeling-Scott L, Papandreou D. Factors associated with delayed initiation and cessation of breastfeeding among working mothers in Abu Dhabi, the United Arab Emirates. *Int J Womens Health.* (2021) 13:539. doi: 10.2147/IJWH.S303041
23. Taha Z, Ali Hassan A, Wikkeling-Scott L, Eltoum R, Papandreou D. Assessment of hospital rooming-in practice in Abu Dhabi, United Arab Emirates: a cross-sectional multi-center study. *Nutrients.* (2020) 12:2318. doi: 10.3390/nu12082318
24. Amin T, Hablas H, Al Qader AA. Determinants of initiation and exclusivity of breastfeeding in Al Hassa, Saudi Arabia. *Breastfeed Med.* (2011) 6:59–68. doi: 10.1089/bfm.2010.0018
25. Hegazi MA, Allebdi M, Almohammadi M, Alnafie A, Al-Hazmi L, Alyoubi S. Factors associated with exclusive breastfeeding in relation to knowledge, attitude and practice of breastfeeding mothers in Rabigh community, Western Saudi Arabia. *World J Pediatr.* (2019) 15:601–9. doi: 10.1007/s12519-019-00275-x
26. Kim B-Y. Factors that influence early breastfeeding of singletons and twins in Korea: a retrospective study. *Int Breastfeed J.* (2017) 12:4. doi: 10.1186/s13006-016-0094-5
27. Ogbo FA, Dhami MV, Awosemo AO, Olusanya BO, Olusanya J, Osuagwu UL, et al. Regional prevalence and determinants of exclusive breastfeeding in India. *Int Breastfeed J*. (2019) 14:20. doi: 10.1186/s13006-019-0214-0
28. Piro SS, Ahmed HM. Impacts of antenatal nursing interventions on mothers breastfeeding self-efficacy: an experimental study. *BMC Pregnancy Childbirth.* (2020) 20:19. doi: 10.1186/s12884-019-2701-0
29. Salim YM, Stones W. Determinants of exclusive breastfeeding in infants of six months and below in Malawi: a cross sectional study. *BMC Pregnancy Childbirth*. (2020) 20:472. doi: 10.1186/s12884-020-03160-y
30. Um S, Chan YZC, Tol B, Sopheab H. Determinants of exclusive breastfeeding of infants under six months among Cambodian mothers. *J Pregnancy.* (2020) 2020:2097285. doi: 10.1155/2020/2097285
31. Smith HA. J OBH, Kenny LC, Kiely M, Murray DM, Leahy-Warren P. Early life factors associated with the exclusivity and duration of breast feeding in an Irish birth cohort study. *Midwifery.* (2015) 31:904–11. doi: 10.1016/j.midw.2015.04.015
32. Panaviene J, Zakharchenko L, Olteanu D, Cullen M, EL-Khuffash A. Factors contributing to non-exclusive breastfeeding in Primigravid mothers. *Ir Med J.* (2019) 112:1003.
33. Stuebe AM, Grewen K, Meltzer-Brody S. Association between maternal mood and oxytocin response to breastfeeding. *J Womens Health.* (2013) 22:352–61. doi: 10.1089/jwh.2012.3768
34. Al Juaid DA, Binns CW, Giglia RC. Breastfeeding in Saudi Arabia: a review. *Int Breastfeed J.* (2014) 9:1. doi: 10.1186/1746-4358-9-1
35. WHO. World Health Organization. *Global nutrition targets 2025: breastfeeding policy brief*. Geneva: World Health Organization (2014).
36. Fikri M, Farid S. *United Arab Emirates family health survey*. Abu Dhabi: Ministry of Health Abu Dhabi. (2000).
37. Taha Z. Trends of breastfeeding in the United Arab Emirates (UAE). *Arab J Nutr Exerc.* (2017) 3:152—9. doi: 10.18502/ajne.v2i3.1356
38. Al-Nuaimi N, Katende G, Arulappan J. Breastfeeding trends and determinants: implications and recommendations for gulf cooperation council countries. *Sultan Qaboos Univ Med J.* (2017) 17:e155. doi: 10.18295/squmj.2016.17.02.004
39. Salem O. *FNC Passes Mandatory Breastfeeding Clause for Child Rights Law*. Abu Dhabi: The National; Abu Dhabi Media Dhabi (2014). Available online at: https://www.thenationalnews.com/uae/government/fnc-passes-mandatory-breastfeeding-clause-for-child-rights-law-1.250017
40. Al Ghazal H, Rashid S, Ruf E. The Sharjah baby-friendly campaign: a community-based model for breastfeeding promotion, protection, and support. *Breastfeed Med.* (2015) 10:437–41. doi: 10.1089/bfm.2015.0095
41. Taha Z, Garemo M, Nanda J. Patterns of breastfeeding practices among infants and young children in Abu Dhabi, United Arab Emirates. *Int Breastfeed J.* (2018) 13:1–10. doi: 10.1186/s13006-018-0192-7

42. Ayed A. Knowledge, attitude and practice regarding exclusive breastfeeding among mothers attending primary health care centers in Abha city. *Int J Med Sci Public Health.* (2014) 3:1355. doi: 10.5455/ijmsph.2014.140820141
43. Hamade H, Naja F, Keyrouz S, Hwalla N, Karam J, Al-Rustom L, Nasreddine L. Breastfeeding knowledge, attitude, perceived behavior, and intention among female undergraduate university students in the Middle East: the case of Lebanon and Syria. *Food Nutr Bull.* (2014) 35:179–90. doi: 10.1177/156482651403500204
44. Khassawneh M, Khader Y, Amarin Z, Alkafajei A. Knowledge, attitude and practice of breastfeeding in the north of Jordan: a cross-sectional study. *Int Breastfeed J.* (2006) 1:17. doi: 10.1186/1746-4358-1-17
45. WHO. *Obesity: preventing and managing the global epidemic: report of a WHO consultation*. Geneva: World Health Organization (2000).
46. Gender Inequality Index (GII) [Internet]. (2019). Available online at: http://hdr.undp.org/en/content/gender-inequality-index-gii (accessed February 2022).
47. *UAE: The sky is the limit for gender reform* [press release]. World Bank Blogs (2020). Available online at: https://blogs.worldbank.org/arabvoices/gender-reforms-united-arab-emirates
48. Karim F, Khan ANS, Tasnim F, Chowdhury MAK, Billah SM, Karim T, et al. Prevalence and determinants of initiation of breastfeeding within one hour of birth: An analysis of the Bangladesh Demographic and Health Survey, 2014. *PLoS ONE.* (2019) 14:e0220224. doi: 10.1371/journal.pone.0220224
49. Chimoriya R, Scott JA, John JR, Bhole S, Hayen A, Kolt GS, et al. Determinants of Full breastfeeding at 6 months and any breastfeeding at 12 and 24 months among women in Sydney: findings from the HSHK Birth Cohort Study. *Int J Environ Res Public Health.* (2020) 17:5384. doi: 10.3390/ijerph17155384
50. Ayesha U, Mamun A, Sayem MA, Hossain MG. Factors associated with duration of breastfeeding in Bangladesh: evidence from Bangladesh demographic and health survey 2014. *BMC Public Health.* (2021) 21:1758. doi: 10.1186/s12889-021-11804-7
51. Al-Matary AAJ. The impact of child rearing by maids on mother child attachment. *Hamdan Med J.* 2013;6:197–204. doi: 10.7707/hmj.v6i2.119
52. Radwan H, Mussaiger AO, Hachem F. Breast-feeding and lactational amenorrhea in the United Arab Emirates. *J Pediatr Nurs.* (2009) 24:62–8. doi: 10.1016/j.pedn.2007.09.005
53. Moore ER, Bergman N, Anderson GC, Medley N. Early skin-to-skin contact for mothers and their healthy newborn infants. *Cochrane Database Syst Rev.* (2016) 11:Cd003519. doi: 10.1002/14651858.CD003519.pub4
54. Conde-Agudelo AD-RJLBJM. Kangaroo mother care to reduce morbidity and mortality in low birthweight infants. *Birth.* (2003) 30:133–4. doi: 10.1111/j.1523-536X.2003.00233.x
55. Rukindo M, Tumwebaze M, Mijumbi EM. First hour initiation of breast feeding & associated factors, among mothers at post natal ward in fort portal referral hospital, Uganda. *Open J Epidemiol.* (2021) 11:15. doi: 10.4236/ojepi.2021.111001
56. Flacking R, Nyqvist KH, Ewald U. Effects of socioeconomic status on breastfeeding duration in mothers of preterm and term infants. *Eur J Public Health.* (2007) 17:579–84. doi: 10.1093/eurpub/ckm019
57. Hunegnaw MT, Gelaye KA, Ali BM. Factors associated with the time to cessation of breastfeeding among mothers who have index children aged two to three years in Debre Markos, northwest Ethiopia: a retrospective follow up study. *BMC Pediatr.* (2018) 18:77. doi: 10.1186/s12887-018-1012-3
58. Economou M, Kolokotroni O, Paphiti-Demetriou I, Kouta C, Lambrinou E, Hadjigeorgiou E, et al. The association of breastfeeding self-efficacy with breastfeeding duration and exclusivity: longitudinal assessment of the predictive validity of the Greek version of the BSES-SF tool. *BMC Pregnancy Childbirth.* (2021) 21:421. doi: 10.1186/s12884-021-03878-3
59. Kakute PN, Ngum J, Mitchell P, Kroll KA, Forgwei GW, Ngwang LK, et al. Cultural barriers to exclusive breastfeeding by mothers in a rural area of Cameroon, Africa. *J Midwifery Womens Health.* (2005) 50:324–8. doi: 10.1016/j.jmwh.2005.01.005
60. Nafee Elsayed HM, Al-Dossary LA. Exclusive breastfeeding, prevalence and maternal concerns: Saudi and Egyptian mothers. *J Educ Pract.* (2016) 7:5–11.
61. Knight-Agarwal CR, Rickwood P, To S, Jani R. The relationship between maternal pre-pregnancy body mass index and exclusive breastfeeding initiation: findings from an Australian obstetric cohort. *Obes Res Clin Pract.* (2021) 15:33–6. doi: 10.1016/j.orcp.2021.01.002
62. Ozenoglu A, Sokulmez Kaya P, Asal Ulus C, Alakus K. The relationship of knowledge and breastfeeding practice to maternal BMI. *Ecol Food Nutr.* (2017) 56:152–70. doi: 10.1080/03670244.2016.1275604

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Validation Study of the Estimated Glycemic Load Model Using Commercially Available Fast Foods

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The recent popularization of low-glycemic foods has expanded interest in glycemic index (GI) not only among diabetic patients but also healthy people. The purpose of this study is to validate the estimated glycemic load model (eGL) developed in 2018. This study measured the glycemic load (GL) of 24 fast foods in the market in 20 subjects. Then, the transportability of the model was assessed, followed by an assessment of model calibration and discrimination based on model performance. The transportability assessment showed that the subjects at the time of model development are different from the subjects of this validation study. Therefore, the model can be described as transportable. As for the model's performance, the calibration assessment found an x^2 value of 11.607 and a p-value of 0.160, which indicates that the prediction model fits the observations. The discrimination assessment found a discrimination accuracy exceeding 0.5 (57.1%), which confirms that the performance and stability of the prediction model can be discriminated across all classifications. The correlation coefficient between GLs and eGLs measured from the 24 fast foods was statistically significant at 0.712 ($p < 0.01$), indicating a strong positive linear relationship. The explanatory powers of GL and eGL was high at 50.7%. The findings of this study suggest that this prediction model will greatly contribute to healthy food choices because it allows for predicting blood glucose responses solely based on the nutrient content labeled on the fast foods.

Keywords: glycemic index (GI), glycemic load (GL), diet, carbohydrate loading, fast foods

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INTRODUCTION

It has been reported that excessive carbohydrate intake causes obesity and diabetes, which lead to claims that carbohydrate intake should be controlled (1). These trends also expanded interest and research in the low-carbohydrate diet, where carbohydrates account for 45% or lower of the total energy intake, or ultra low-carbohydrate diet, where the percentage is 10% or lower (2). It has been also suggested that it is positive to choose foods with low glycemic index (GI), thereby slowing down digestion and absorption and controlling appetite in the short term, and interest in low-GI foods continues today (3). GI reflects the digestion and absorption speed of carbohydrates in a food. GI is measured by comparing the blood glucose response of a food after consumption with the blood glucose response of the reference foods, and expressed in percentage (4).

Atkinson et al. (5) classified a food with a GI of 55 or lower as a low-GI food, a food with a GI above 55 and below 70 as a medium-GI food, and those with a GI exceeding 70 as a high-GI food. It has been reported that low-GI diet slows down carbohydrate digestion and absorption, increases satiety, and improves blood lipids and insulin resistance, thereby mitigating risks of cardiovascular diseases, diabetes, and obesity (6). The Korean Food Sanitation Act still does not allow GI to be included in processed food labels, whereas in Australia, for example, the Glycemic Index Foundation (GIF) allows for GI labeling on food packaging through the "Low GI Symbol Program" (7).

The recent popularization of low-glycemic foods has expanded interest in GI not only among diabetic patients but also healthy people (8). However, GI does not take single-time carbohydrates intake into account. To address this shortcoming, we need to consider glycemic load (GL) to quantify the glycemic effect included in a single portion of a food (9). A food with a GL of 10 or lower has been classified as a low-GL food, whereas a food with a GL over 10 and below 20 has been classified as a medium-GL food, and a food with a GL of 20 or higher has been classified as a high GL food (10). The type of carbohydrate (potato, bread, rice, etc.) and the food consumed with carbohydrates affects glycemic indicators such as GI and GL (11). Lee et al. (12) developed the estimated glycemic load model (eGL) equation for Koreans, who mostly rely on mixed diets rather than consuming a single food containing carbohydrate, to address the complexity and inaccuracy of glycemic calculation for mixed diets. Subsequently, the diets of Korean adults were evaluated using the data from the Korea National Health and Nutrition Examination Survey (KNHANES) and the developed eGL prediction model, to verify the usefulness of the model (13). As such, this study aims to review the validity of the eGL prediction model calculated using a number of mixed meal replacements.

MATERIALS AND METHODS

Research Design

The survey took from July 15 to September 21, 2019 at Kyung Hee University and Kookmin University under the approval of the Institutional Review Board of Kyung Hee University Global Campus (approval number: KHGIRB-19-147, approval date: June 26, 2019). Subjects were recruited through an open call process and were briefed about the research process during the first visit. Then, the research continued with the subjects who, after reading the research subject manual, voluntarily agreed to participate in the research and signed the research subject consent form. The blood glucose levels of the subjects were measured at 0 min before food consumption, and 15, 30, 45, 60, 90, and 120 min after consumption. All subjects were provided with test food between 7 and 11 a.m., and the intake was completed within 15 min. The measurements were recorded in a blood glucose measurement record sheet. The subjects were arbitrarily divided into two groups, and were visited 13–14 times throughout the research period to measure blood glucose levels.

Subjects

The subjects were selected from healthy adults with normal fasting blood glucose levels and no significant health-related issues, aged between 20 and 45 years. They were recruited by posting a call for subjects indicating the selection and exclusion criteria at the universities. The exclusion criteria included: history of diabetes in immediate family members; chronic diseases or endocrine diseases such as thyroid diseases; pathophysiological risk factors such as digestive disorders; inability to go through self-blood glucose test using a blood glucose tester due to psychological fear; body mass index (BMI) of 25 kg/m^2 or higher (obesity) for Asian defined by World Health Organization/International Association for the Study of Obesity/International Obesity Task Force (WHO/IASO/IOTF) (14). An oral glucose tolerance test (OGTT) was conducted during the first visit, to screen out subjects with a fasting blood glucose level of 100 mg/dL or higher and those with a blood glucose level of 140 mg/dL or higher 2 h after consuming glucose solution. In addition, subjects were excluded if they failed to complete four baseline blood glucose response tests (two with glucose solution, two with steamed white rice) or could not continue participation due to health issues. In accordance with the International Organization for Standardization (ISO) standards (15), blood glucose tests were conducted only on subjects who did not consume alcohol on the previous day, maintained normal diet and sleep, and did not engage in intense exercises on the morning of the visitation day. The subjects were informed of this before each test. In addition, tests were conducted after confirming whether the subjects did not consume any food including water for at least 10 h. ISO technical committees which suggest 10 subjects per food item is recommended for measuring GI.

Research Method

Body Composition Analysis

During the first visit, the body components of the subjects on empty stomach were measured using a body fat analyzer (InBody 270, InBody Co., Ltd.).

Baseline Blood Glucose Response Measurements

The ISO standards clearly specify the standard method for determining the GI of a food. Reference foods were selected based on the ISO standards, and the blood glucose curve response of each subject was measured in advance. A reference food is defined as a food with around 100 GI. In this study, all subjects were instructed to consume the reference foods, and their individual blood glucose response curves were measured. Glucose, white bread, and rice have been proposed as reference foods because they have more standardized carbohydrate content than other foods, and show fewer fluctuations in GI values (15, 16). In addition, the blood glucose response of a reference food is expressed as the incremental area under the blood glucose response curve (IAUC). It is recommended to conduct at least In review two blood glucose response tests on a separate day during the research period. In this study, the following foods were selected as reference foods allowed under the above standards: glucose solution (dextrose, anhydrous, 50 g); and steamed white rice (154 g, glucose content around 50 g), which is a carbohydrate food enjoyed by most Koreans. Then, two tests were conducted for each in accordance with the ISO standards.

Food Consumption and Blood Glucose Measurements

After the baseline blood glucose response tests, subjects consumed the reference foods four times and different processed foods nine to ten times. Each subject was visited 13–14 times. Each food was consumed by eight different subjects, followed by blood glucose measurements. Subjects measured their own blood glucose levels using ACCU-CHEK Performa (Roche Diagnostics Korea Co., Ltd) at 0 min before food intake and 15, 30, 45, 60, 90, and 120 min after food intake. Each subject took his/her blood glucose measurements by washing the hands before the test, completely removing moisture, disinfecting the area with alcohol wipes, and then taking blood from the tip of a finger using the needle of the tester. The subjects were instructed to record their first blood glucose measurements. During the test, the subjects were instructed to refrain from water consumption. If a subject wanted, he/she was allowed to consume 100 mL or less of water. To rule out the effect of blood glucose response, the subjects were instructed to sit and refrain from speaking or making big movements during the 2-h measurement.

Test Food Selection

In order to validate the estimated eGL prediction model developed in a previous study, this study selected 24 prediction models in the market that contain carbohydrate (g), protein (g), fat (g), and dietary fiber (g) based on the nutrient labels. The 24 processed foods were selected to vary the carbohydrate, processed food, fat, and dietary fiber contents. The selected foods included: two types of bread (bulgogi croquette and sponge cake); three types of calorie control foods (balance shake, sweet potato health meal cold/hot, and tofu and lentil rice meal); two types of cereals (cereal and whole-grain cereal); two types of In review dumplings [dumplings with kimchi (frozen) and dumplings with meat (frozen)]; three types of readymade rice [bibimbap (frozen), fried rice with hamburger steak (frozen), and fried rice with shrimp (frozen)]; a type of hot dog (cheese and sausage hot dog); three types of noodles (cream past, spicy noodle, and tomato pasta); two types of porridge (beef and mushroom rice porridge and red bean porridge); a type of salad (corn salad); two types of snacks (dried tofu snack and almond cookies); a type of soup (button mushroom soup); and a type of tteokbokki (wheat noodle tteokbokki). Each food was distributed between the two subject groups. All foods were served after preparing them with microwave ovens in accordance with the instructions indicated on the food packaging. **Table 4** lists the 24 processed foods energy and nutrient values.

GI, GL, and eGL Equations

The blood glucose measurements were analyzed using Graphpad Prism 8.3.0 to calculate their IAUCs. Then, GI (17) and GL (5) were calculated using the IAUC values and the following equations.

$$GI = \textit{IAUC after consuming the processed foods} \div \textit{IAUC after consuming glucose solution} \times 100$$

$$GL = GI \times \textit{available carbohydrate}(\textit{carbohydrate} - \textit{dietary fiber}) \div 100$$

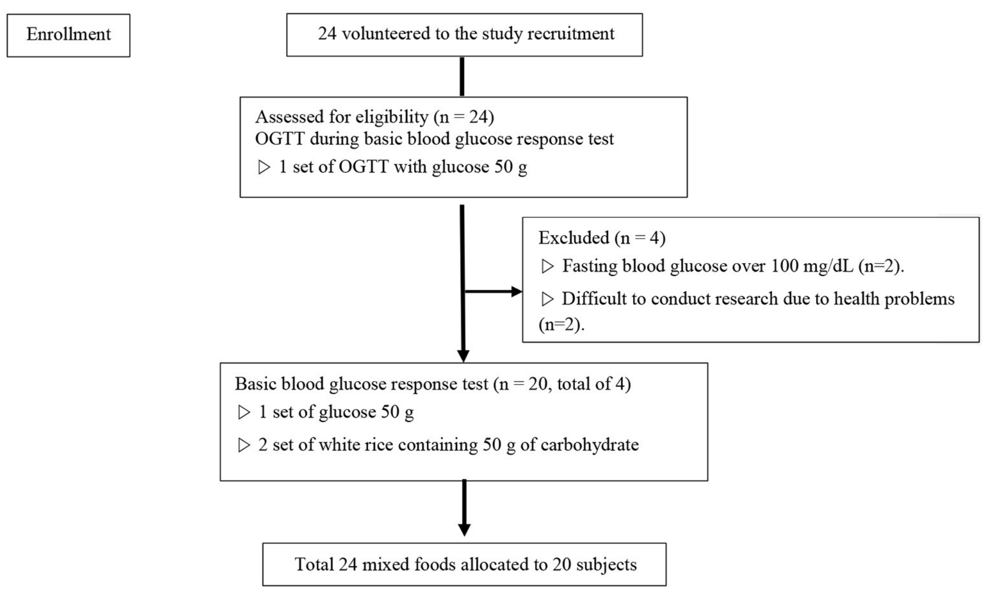

FIGURE 1 | Flow chart of study process.

The carbohydrate, dietary fiber, prediction model, fat, and other nutrient content in **Table 3** were applied to the eGL prediction model equation (13) to calculate eGLs.

$$eGL = a + [b \times (carbohydrate - dietary\ fiber)] - (c \times fat) - (d \times protein \times protein) - (e \times dietary\ fiber \times dietary\ fiber)$$

Statistical Analysis

The validation of a prediction model hinges on how well the model works for the subjects who did not participate at the time of the model's development (18). To validate the model, the transportability of the model was assessed, followed by an assessment of model calibration and discrimination based on model performance. Model transportability assessment is a method to verify whether the validity of the model can be ensured with different subjects, research period, and research organizations. It has been reported that transportability can be only assessed in external validation but is not always included in the assessment (19, 20). After the transportability assessment, the performance of the model was assessed to understand how suitable the prediction model is for application to new subjects (21). First, through the transportability assessment, the kai square test and the independent sample T-test were used to verify the general characteristics of the two group and the difference between the two groups in terms of the factors included in the eGL prediction model. In addition, the logistic regression analysis was used to calculate the regression coefficients and standard errors of the factors included in the model and verify their calibration. The calibration assessment verifies how closely the values predicted using a prediction model match the observed values. The discrimination assessment indicates how the observed values and the predicted values are discriminated in sub-groups, not the overall subjects. In the calibration assessment, the subjects were classified based on their genders, BMIs, and body fat for the Hosmer-Lemeshow goodness-of-fit test. Lastly, the eGLs, the observed Gls, and the area under a receiver operating characteristic curve (AUROC) were used to calculate the confidence interval and confirm the model's discrimination. Then, the IAUC, GI, GL, and eGL values measured from the subjects were expressed as means and standard deviations (SD). In addition, the Pearson correlation analysis was used to see whether the observed Gls are correlated with the eGLs. In addition, the simple linear regression analysis was used to numerically confirm the effect of various variables on the GL-eGL correlation. All collected data were analyzed using IBM SPSS Statistics version 25, and the findings were tested for significance at a significance level of $p < 0.05$.

RESULTS

General Characteristics of the Subjects

For this study, 24 subjects were recruited that fit the selection criteria through an open call process. Four of them were excluded during the first using through an OGTT, and the research was conducted on the remaining 20 subjects (**Figure 1**). **Table 1** indicates the general characteristics of the subjects and the mean nutrient contents of the foods used for the study. Each item is indicated in both the mean and the SD. The subjects consisted of 20 healthy adults (ten men, ten women). The mean BMI was at normal weight category at 21.8 ± 1.92 kg/m². The waist-hip ratio of the subjects was 0.8 ± 0.0, which is within the normal level (cut-offs of waist–hip ratio for the risk for abdominal obesity is male≥0.90, female≥0.85) (23). The subject's mean fasting blood glucose (measured after 10 h of fasting or longer) was within the normal range at 92.8 ± 4.78 mg/dL. The available carbohydrate, fat, protein, and fiber in all test foods used in the study were 37.9 ± 17.65 g, 10.2 ± 8.14 g, 8.5 ± 5.81 g, and 2.8 ± 2.86 g, respectively.

TABLE 1 | Comparison of characteristics between validation subjects and development subjects for eGL prediction model.

	Current validation study	Previously developed eGL prediction model[c]	*P*-value
Characteristics of subjects			
N	20	34	
Data collection time	July to September, 2019	April to August, 2017	
Men (%)	50	50	0.364
Age	24.3 ± 1.98	23.2 ± 2.11	0.052
Height (cm)	169.0 ± 7.69	168.6 ± 7.27	0.841
Weight (kg)	62.7 ± 9.10	64.8 ± 11.68	0.487
BMI (kg/m^2)[a]	21.8 ± 1.92	22.7 ± 3.44	0.289
Skeletal muscle mass (kg)	27.4 ± 6.32	26.9 ± 6.27	0.749
Percent body fat (%)	22.0 ± 7.69	21.3 ± 9.33	0.762
Waist-hip ratio	0.8 ± 0.04	0.8 ± 0.05	0.392
Basal metabolism (kcal)	1,431.9 ± 221.15	1,416.4 ± 221.95	0.805
/blood glucose (mg/dL)	92.8 ± 4.78	92.7 ±5.05	0.866
Estimated regression coefficient			
N	192	239	
Available carbohydrate[b]	37.9 ± 17.65	47.6 ± 20.32	0.000
Fat	10.2 ± 8.14	9.4 ± 6.27	0.249
Protein	8.5 ± 5.81	11.6 ± 6.47	0.000
Fiber	2.8 ± 2.86	4.6 ± 3.34	0.000

Characteristics of subjects represent M ± SD.
[a]Body Mass Index.
[b]Total carbohydrate-dietary fiber.
[c]Lee (22).

eGL Prediction Model Transportability

Table 1 shows the transportability assessment results of the eGL prediction model. There was no difference in terms of height (cm), weight (kg), BMI (kg/m^2), skeletal muscle mass (kg), or percent body fat (%) between the subjects for validation and the subjects at the time of the model's development. On the other hand, the 24 foods selected for this validation study had significantly different nutrient contents from the 32 foods

TABLE 2 | Hosmer-Lemeshow goodness of fit test and ROC curve for eGL prediction model.

		H-L test		C-statistic (95% CI)
		x^2	*P*	
Overall		11.607	0.160	0.571 (0.400–0.741)
Gender	Men	7.655	0.468	0.521 (0.052–0.991)
	Woman	9.427	0.308	0.589 (0.420–0.757)
BMI (kg/m^2)[a]	≤23 (normal)	16.498	0.036	0.564 (0.400–0.727)
	>23 (overweight, obesity)	7.571	0.476	0.564 (0.068–1.000)
Percent body fat (%)	Average	11.608	0.170	0.543 (0.362–0.724)
	above average	1.088	0.998	0.783 (0.672–0.894)

[a]*Body Mass Index.*

TABLE 3 | Relationships between means of GL and eGL for available processed food.

		GL	eGL
GL[a]	Pearson's correlation	1	0.712**
	P-value	–	0.000
eGL[b]	Pearson's correlation	0.712**	1
	P-value	0.000	–

***Values are significant in both sides (P < 0.01).*
[a]*Glycemic index.*
[b]*Glycemic load.*

selected for the model development, except for fat (10.2 vs. 9.4 g, $p = 0.000$). Specifically, the foods used for this validation study had less protein than the foods used for model development (8.5 vs. 11.6 g, $p = 0.000$), less fiber (2.8 vs. 4.6 g, $p = 0.000$), and less carbohydrate (37.9 vs. 47.6 g, $p = 0.000$). The logistic regression analysis confirmed that the regression coefficient included in the eGL prediction model matched the coefficient at the time of the model's development (**Table 1**).

Calibration and Discrimination Assessments for the eGL Prediction Model

The Hosmer-Lemeshow goodness-of-fit test for calibration assessment and the AUROC test for discrimination assessment resulted in the findings listed in **Table 2**. Across all subjects, the x^2 value was 11.607 and the p-value was 0.160. Between genders, the men reported an x^2 value of 7.655, and a p-value of 0.468, whereas the x^2 value was 9.427 and the p-value was 0.308 for the women. As for BMI, the x^2 value was 16.498 and the p-value was 0.036 in people with normal BMIs (18.5–23 kg/m^2), whereas the same values were 7.571 and 0.476 among overweight and obese people (BMI exceeding 23 kg/m^2). As for percent body fat (%), the x^2 value was 11.608 and the p-value was 0.170 among subjects with standard body fat, and the same values were 1.088 and 0.998 among people with higher than standard body fat (**Table 2**).

The discrimination of the model was assessed using the observed GL values, the eGL values from the prediction model, and the AUROC, at a confidence interval of 95%. The AUROC for all subjects was 0.571 (95% CI = 0.400–0.741), and 0.521 for men (95% CI = 0.052–0.991) and 0.589 for women (95% CI = 0.420–0.757). As for BMI, the AUROC was 0.564 (95% CI = 0.400–0.727) in the 23 kg/m^2 or lower group (normal weight), which was the same for subjects with BMIs exceeding 23 kg/m^2 (overweight). As for percent body fat, the AUROC was 0.543 (95% CI = 0.362–0.724) and 0.783 (95% CI = 0.672–0.894), respectively. The AUROC was the highest among participants with standard or higher percent body fat, and lowest among male subjects at 0.521 (**Table 2**).

Correlation Between GL and eGL

Table 3 summarizes the findings on the GL-eGL correlation of the 24 fast foods. The correlation coefficient was statistically significant at 0.712 ($p < 0.01$), indicating a strong positive correlation. **Figure 2** indicates the simple regression analysis results for GL and eGL of the 24 processed foods. According to the simple regression model, $measure\ GL = -9.27 + 1.64 \times estimated\ GL$measured GL. The findings were significant at a $p < 0.001$ significance level. The eGL was found to explain 50.7% of the measured GL. In other words, when eGL increases by 1, actual GL increases by 1.637 (**Table 3**).

GI and GL by Food

Table 4 lists the IAUC, GI, GL, and eGL calculated based on the dietary intake and nutrient contents of the processed foods used in the study and the blood glucose measurements. Among the 24 food products, 15 products were low-GI foods (GI ≤ 55), three products were high-GI foods (GI ≥ 70), and the other six products were medium-GI foods (55 < GI < 70). The low-GI foods were: two bread products (bulgogi croquette and sponge cake); one hot dog product (cheese and sausage hot dog); three noodle products (cream pasta, spicy noodle, and tomato pasta); one salad product (corn salad); one shake product (balance shake); one soup product (button mushroom soup); and one tteokbokki product (wheat noodle tteokbokki). Bibimbap (frozen), fried rice with shrimp (frozen), and cereals were high-GI foods. Among the 24 food products, six products were low-GL foods (GL ≤ 10), 12 products were high-GL foods (GL ≥ 20), and the other six products were medium-GL foods (10 < GL < 20). Button mushroom soup, corn salad, spicy noodle, balance shake, cheese and sausage hot dog, and sponge cake were low-GL foods (**Table 4**).

Table 5 summarizes the classifications based on GI and GL measurements from the foods selected for this study. Corn salad, button mushroom soup, spicy noodle, balance shake, cheese and sausage hot dog, and sponge cake were classified as low-GI and low-GI foods. Beef and mushroom rice porridge and whole-grain cereal were classified as medium-GI and medium-GL products. Bibimbap (frozen), fried rice with shrimp (frozen), and cereals were high-GI and high GL foods. Low-GI and high-GL foods included cream pasta, almond cookies, tomato pasta, fried rice with hamburger steak (frozen), and wheat noodle tteokbokki. Medium-GI and high-GL foods were dried tofu snack, red bean porridge, dumplings with meat (frozen), and tofu and lentil rice meal.

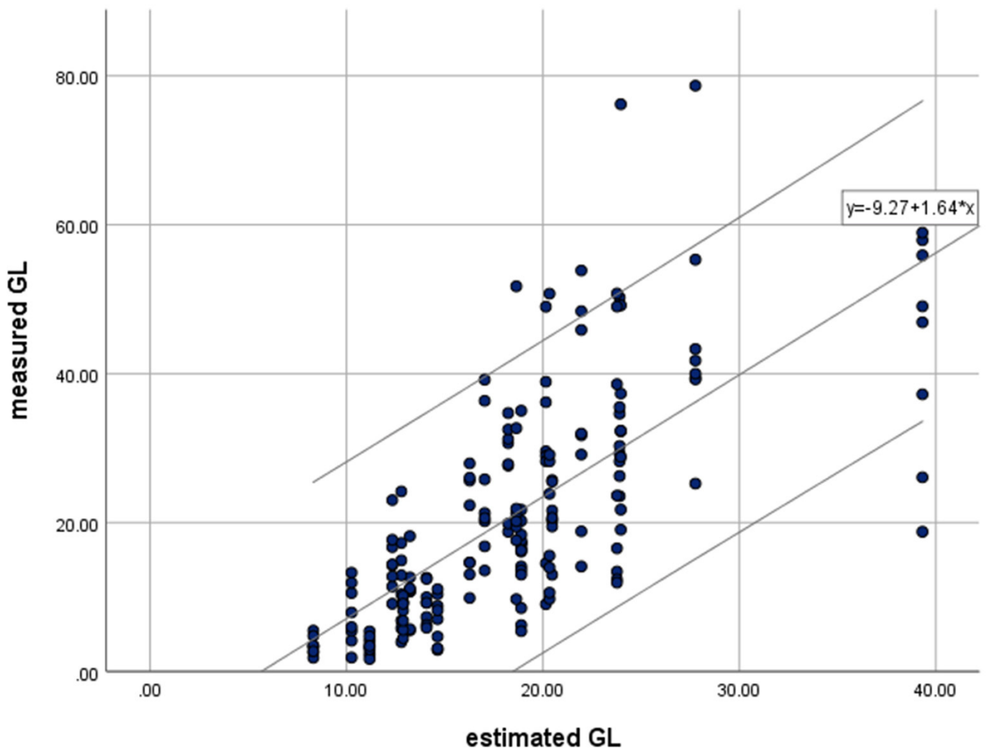

FIGURE 2 | Relationships between means of measured glycemic load (GL) and estimated glycemic load (eGL) for available processed food by simple linear regression. Regression were made for all test meals (• and - : $R^2 = 0.507$, $P = 0.000$). Values of parameter estimation.

DISCUSSION

This study was conducted to validate the eGL prediction model developed in a previous study (12) by commercially available fast foods with a more diverse nutrient content, assessing the model's prediction model and performance, and using correlation analysis. The transportability assessment showed that the subjects at the time of model development had different characteristics from those of this validation study. Therefore, the model can be described as transportable. As for the performance assessment of the eGL model across all subjects, the calibration assessment found the good fit of the model. The discrimination of the prediction model was assessed at 0.571 (95% CI: 0.400–0.741). Although it is not highly accurate, as the value exceeds 0.5, the finding indicates the possibility of validating the performance and stability of the prediction model. The correlation analysis between the observed GL and the eGL across the 24 fast foods used in this study found a correlation coefficient of 0.712 and statistical significance at a significance level of 0.01, which indicates a strong positive correlation. The finding suggests that it is appropriate to use the eGL prediction model to predict GL.

The transportability of the prediction model was analyzed based on the research data at the time of the model's development. As widely recommended for transportability assessment, the characteristics of the validation subjects and those of the development subjects were directly compared (21). The body measurement items of the validation subjects and the development subjects were not significantly different, which can be attributed to the fact that healthy subjects with less blood glucose response fluctuations were selected for both studies for higher accuracy, as typically recommended for blood glucose studies (16). However, the available carbohydrate, protein, and fiber content were different between the development study and this validation study, which indicates the generalizability of the developed model. Therefore, the model can be described as transportable.

The x^2 values from the Hosmer-Lemeshow test indicate the goodness-of-fit of the model, which shows the congruence between the actually observed dependent variables and the predictions from the model (18). An x^2 value close to 0 indicates a higher level of goodness-of-fit. The model is statistically significant if the significance probability is higher than a significance level of 0.05(24, 25). Across all subjects, the x^2 value was 11.607 and the p-value was 0.160, indicating that the prediction model is a good fit with the observed values. Between genders, the men reported an x^2 value of 7.655, and a p-value of 0.468, whereas the x^2 value was 9.427 and the p-value was 0.308 for the women. The observed GLs were congruent to the eGLs under both classifications, however, men group had higher level of goodness-of-fit than women. As for BMI, the x^2 value was 16.498 and the p-value was 0.036 in people with normal BMIs (18.5–23 kg/m^2), which means the prediction model was

TABLE 4 | Nutrient values, IAUC, GI, GL, and eGL values of fast foods used in this study.

Category	Food name (kcal/serving)	Carbohydrate (g)	Dietary fiber (g)	Protein (g)	Fat (g)	IAUC[a]	GI[b]	GI sort	GL[c]	GL sort	eGL
Bread	Bulgogi croquette (220 kcal/80 g)	25	2	9	10	1,843 ± 775	52 ± 29	Low	12 ± 7	Med	13
	Sponge cake (105 kcal/30 g)	18	0	2	2.8	1,636 ± 412	44 ± 11	Low	8 ± 2	Low	13
Calorie controlled meal	Balance shake (230 kcal/60 g)	31	9	20	5	1,304 ± 758	35 ± 18	Low	8 ± 4	Low	10
	Sweet potato healthy meal (Cold) (185 kcal/150 g)	34	0	4	3.8	2,767 ± 969	52 ± 23	Low	18 ± 8	Med	19
	Sweet potato healthy meal (Hot) (185 kcal/150 g)	34	0	4	3.8	2,348 ± 1,077	42 ± 17	Low	14 ± 6	Med	19
	Tofu lentil-rice meal (340 kcal/210 g)	50	0	19	8	2,503 ± 816	68 ± 28	Med	34 ± 14	High	22
Cereal	Cereal (150 kcal/40 g)	35	0	2	0	3,026 ± 976	83 ± 37	High	29 ± 13	High	20
	Whole-grain cereal (169 kcal/40 g)	30	1.9	2.9	4.7	2,514 ± 612	69 ± 25	Med	19 ± 7	Med	16
Dumpling	Dumplings with kimchi; frozen (407.5 kcal/220 g)	40	5.5	15.5	22.0	1,749 ± 713	31 ± 12	Low	11 ± 4	Med	13
	Dumplings with meat; frozen (467.5 kcal/200 g)	50	1.5	19.5	21.5	3,643 ± 1,172	58 ± 12	Med	28 ± 6	High	18
Easy cooked rice	Bibimbap; frozen (315 kcal/217 g)	58	7	6	8	4,406 ± 2,319	73 ± 36	High	37 ± 18	High	24
	Fried rice with hamburger steak; frozen (535 kcal/275 g)	69	7	14	24	3,258 ± 1,103	52 ± 13	Low	32 ± 8	High	24
	Fried rice with shrimp; frozen (375 kcal/225 g)	63	2	7	11	3,880 ± 1,299	74 ± 26	High	45 ± 16	High	28
Hot dog	Cheese and sausage hot dog (230 kcal/80 g)	28	2	6	11	1,885 ± 518	35 ± 10	Low	9 ± 3	Low	14
Noodle	Cream pasta (560 kcal/331.2 g)	58	2	16	30	1,429 ± 413	37 ± 7	Low	21 ± 4	High	20
	Spicy noodle (135 kcal/186.5 g)	25	2	1	3.7	1,127 ± 550	31 ± 14	Low	7 ± 3	Low	15
	Tomato pasta (290 kcal/270 g)	53	4	10	5	1,901 ± 809	55 ± 34	Low	27 ± 17	High	24
Porridge	Beef and mushroom rice porridge (155 kcal/250 g)	26	3	7	13	2,451 ± 700	65 ± 19	Med	15 ± 4	Med	12
	Red bean porridge (205 kcal/250 g)	46	10	9	0.5	2,589 ± 1,325	68 ± 35	Med	24 ± 13	High	19
Salad	Corn salad (100 kcal/115 g)	18	3	2	4.8	1,172 ± 315	23 ± 8	Low	4 ± 1	Low	11
Snack	Almond cookies (420 kcal/80 g)	48	0	8	22	1,757 ± 939	47 ± 28	Low	23 ± 14	High	20
	Dried tofu snack (310 kcal/65 g)	36	0	6	16	2,445 ± 554	67 ± 25	Med	24 ± 9	High	17
Soup	Button mushroom soup (165 kcal/190 g)	13	2	4	11	1,135 ± 194	31 ± 11	Low	3 ± 1	Low	8
Tteokbokki	Wheat noodle tteokbokki (430 kcal/140 g)	91	3.3	11.1	3	2,716 ± 1,134	50 ± 17	Low	44 ± 15	High	39

Values of IAUC, GI, GL represent M ± SD.
[a]*Incremental area under the blood glucose response curve.*
[b]*Glycemic index.*
[c]*Glycemic load.*
In the GI sort, low GI foods (GI ≤ 55) were shown as "Low," moderate GI foods (55 < GI < 70) as "Med," and high GI foods (GI ≥ 70) as "High".
In the GL sor, low GL foods (GL ≤ 10) were represented as "Low," moderate GL foods (10 < GL < 20) as "Med," and high GL foods (GL ≥ 20) as "High".

not statistically significant. The same values were 7.571 and 0.476 among overweight and obese people (BMI exceeding 23 kg/m^2), indicating that the model is a good fit. As for percentage of body fat, the prediction models were found to be statistically significant in both groups. However, Percent body fat above average group had higher goodness-of-fit in the prediction models than percent body fat average group according to x^2.

It has been suggested that an AUROC of "0.5 or higher and below 0.7" indicates low accuracy, "0.7 or higher and below 0.9" indicates medium accuracy, and "0.9 or higher and below 1.0" indicates high accuracy. A higher AUROC value indicates a higher level of discrimination (21, 26). The AUROC for all subjects was 0.571 (95% CI = 0.400–0.741), which indicates low discrimination accuracy at 57.1%. The discrimination accuracy was similar between the two genders: 52.1% for men and 58.9% for women. As for the classifications based on BMI, in both below 23 or above 23, discrimination accuracy was at 56.4%. As for percent body fat, the group with standard body fat reported a discrimination accuracy of 54.3%, whereas it was 78.3% for the subjects with higher-than-standard body fat. The prediction model was found to be less accurate across all subjects, and consistent findings were observed across genders, BMI groups, and body fat groups. The AUROC assessment found a discrimination accuracy exceeding 0.5 across all classifications,

TABLE 5 | Classification between measured GI and GL for one serving of provided food.

		GI classification		
		Low (GI ≤ 55)	**Medium (55 < GI < 70)**	**High (GI ≥ 70)**
GL classification	Low (GL ≤ 10)	Button mushroom soup Corn salad Spicy noodle Balance shake Sponge cake Cheese and sausage hot dog	–	–
	Medium (10 < GI < 20)	Dumplings with kimchi; frozen Bulgogi croquette Sweet potato healthy meal (Hot) Sweet potato healthy meal (Cold)	Beef and mushroom rice porridge Whole-grain cereal	–
	High (GI ≥ 20)	Cream pasta Almond cookies Tomato pasta Fried rice with hamburger steak; frozen Wheat noodle tteokbokki	Dried tofu snack Red bean porridge Dumplings with meat; frozen Tofu lentil-rice meal	Cereal Bibimbap; frozen Fried rice with shrimp; frozen

which confirms that the performance and stability of the prediction model can be discriminated across all classifications.

The correlation coefficient between GLs and eGLs measured from the 24 processed foods was statistically significant at 0.712 ($P < 0.01$), indicating a strong positive linear relationship. Kim et al. (27) applied foods' nutrient contents and GL measurements to the GL to compare GL and eGL values. They found a correlation coefficient of 0.866 indicating a strong and statistically significant ($p < 0.01$) positive correlation. The findings suggest that GL measurements can be predicted by applying the nutrient contents from other previous studies to the eGL model.

Assuming that the classifications for eGL are the same as the GL classifications, balance shake and button mushroom soup were classified as low-GL foods. They were also classified as low-GL foods in the estimated GL prediction model. Bulgogi croquette, sweet potato healthy meal (cold/hot), whole-grain cereal, dumplings with kimchi (frozen), and beef and mushroom rice porridge were found to have medium GL. These foods were also classified as medium-GL foods in the estimated GL prediction model. Tofu and lentil rice meal, cereal, bibimbap (frozen), fried rice with hamburger steak (frozen), fried rice with shrimp (frozen) cream pasta, tomato pasta, almond cookies, and wheat noodle tteokbokki were found to have high GL. These foods were also classified as high-GL foods in the estimated GL prediction model. Sweet potato healthy meal (cold/hot), dried tofu snack, and cereals were found to have different GIs depending on their protein, fat, and fiber content, despite the fact that their carbohydrate contents are similar. These findings are similar to those reported by Sun et al. (28), who reported that consuming white rice, oil 30 g, chicken protein 20 g, and vegetable 120 g results in lower blood glucose response and GI than consuming white rice (with available carbohydrate of 50 g). The findings are also similar to those reported by Quek et al. (29), who reported a significant decline in blood glucose response when consuming white rice with tofu (bean protein).

High-GI and high-GL foods are digested and absorbed faster and create faster blood glucose response, resulting in a rapid increase in early blood glucose levels (30). Therefore, caution is advised when consuming these foods. In addition, the GI ≤ 55 and 55 < GI < 70 sections and the GL ≤ 10 and 10 < GL < 20 sections indicate low-GI foods, low-GL foods (3), and medium-GL/medium-GI foods, which are characterized by slower digestion and absorption and slower blood glucose response, and can be safely consumed.

The findings of this study confirmed a strong correlation between eGL values and GL measurements based on available carbohydrate, protein, fat, and fiber. This suggests that GL values can be predicted using the eGL prediction model and food nutrient contents, instead of repeatedly taking blood glucose measurements. As such, the model is expected to contribute to facilitating GL measurement. The model is expected to be particularly helpful for Koreans by providing quantitative and qualitative information on carbohydrate intake. Our model provides accurate information on the GLs of the foods recently preferred by Koreans, who tend to eat out more and consume more fast foods. The information will be useful for patients and people requiring weight control, and contribute to prevention and management of chronic diseases.

As for the limitation of this study, first, although using finger-prick glucose test is a well-established procedure, used widely in hospitals as a standard practice, there is still a possibility of low accuracy by measuring blood sugar by self-blood glucose meter. Second, the subject criteria for the validity verification study and the subject criteria at the time for the development of the prediction model were similar, which is not the best choice for an external feasibility study.

To our knowledge this is the first study not only in Korea, but, also globally that suggest a simplified blood glucose prediction model. This equation, especially, may serve as a convenient blood glucose management method for diabetic patients, people with impaired glucose tolerance, and people seeking prevention and management of chronic diseases. We can assure that this model facilitates GL prediction, and promotes understanding of blood glucose control among people in need of, or interested in, blood glucose control, and helps people with their food choice and health management in general.

CONCLUSION

With the increase of consumers purchasing fast foods compared to home-cooked meals, the findings of this study suggest that this prediction model will greatly contribute to healthy food choices because it allows customers to predict blood glucose responses based on the readily available nutrient label.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by KyungHee University, Global Campus IRB. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YP, S-JC, and KN conceptualized and designed the study. ML, KN, and S-JC selected the test foods and conducted the statistical analyses. ML and YP recruited participants, performed the experiment, and prepared the original draft. HK Interpretation of the data and prepared the manuscript in English. ML, HK, and YP finalized the manuscript. All authors listed have made substantial, direct, intellectual contribution to the work and approved it for publication.

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REFERENCES

1. Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, et al. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. *Lancet Public Health.* (2018) 3:e419–28. doi: 10.1016/S2468-2667(18)30135-X
2. Lee M, Kim H. A minireview on carbohydrate in weight management diet: the quantity and the quality. *J Korean Med Obes Res.* (2005) 5:121–31. Available online at: https://www.koreascience.or.kr/article/JAKO200503039786504.page
3. Sun F, Li C, Zhang Y, Wong SH, Wang L. Effect of glycemic index of breakfast on energy intake at subsequent meal among healthy people: a meta-analysis. *Nutrients.* (2016) 8:37. doi: 10.3390/nu8010037
4. Kim IJ. Glycemic index revisited. *Korean Diabetes J.* (2009) 33:261–6. doi: 10.4093/kdj.2009.33.4.261
5. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. *Diabetes Care.* (2008) 31:2281–3. doi: 10.2337/dc08-1239
6. Schwingshackl L, Hobl LP, Hoffmann G. Effects of low glycaemic index/low glycaemic load vs. high glycaemic index/high glycaemic load diets on overweight/obesity and associated risk factors in children and adolescents: A systematic review and meta-analysis. *Nutr J.* (2015) 14:87. doi: 10.1186/s12937-015-0077-1
7. Marinangeli CP, Castellano J, Torrance P, Lewis J, Gall Casey C, Tanuta J, et al. Positioning the value of dietary carbohydrate, carbohydrate quality, glycemic index, and GI labelling to the canadian consumer for improving dietary patterns. *Nutrients.* (2019) 11:457. doi: 10.3390/nu11020457
8. Park M, Chung S, Shim JE, Jang S, Nam K. Effects of macronutrients in mixed meals on postprandial glycemic response. *J Nutr Health.* (2018) 51:31–9. doi: 10.4163/jnh.2018.51.1.31
9. Henry CJK, Lightowler HJ, Strik CM, Renton H, Hails S. Glycaemic index and glycaemic load values of commercially available products in the UK. *Br J Nutr.* (2005) 94:922–30. doi: 10.1079/BJN20051594
10. Venn BJ, Green TJ. Glycemic index and glycemic load: measurement issues and their effect on diet–disease relationships. *Eur J Clin Nutr.* (2007) 61:S122–31. doi: 10.1038/sj.ejcn.1602942
11. Collier GR, Wolever TM, Wong GS, Josse RG. Prediction of glycemic response to mixed meals in noninsulin-dependent diabetic subjects. *Am J Clin Nutr.* (1986) 44:349–52. doi: 10.1093/ajcn/44.3.349
12. Lee H, Um M, Nam K, Chung SJ, Park Y. Development of a prediction model to estimate the glycemic load of ready-to-eat meals. *Foods.* (2021) 10:2626. doi: 10.3390/foods10112626
13. Ha K, Nam K, Song Y. Estimated glycemic load (eGL) of mixed meals and its associations with cardiometabolic risk factors among Korean adults: data from the 2013∼ 2016 Korea National Health and Nutrition Examination Survey. *J Nutr Health.* (2019) 52:354–68. doi: 10.4163/jnh.2019.52.4.354
14. World Health Organization. *The Asia-Pacific Perspective: Redefining Obesity and Its Treatment* (2000).
15. International Organization for Standardization. *Food Products-Determination of the Glycaemic Index (GI) and Recommendation for Food Classification*. Cham: International Organization for Standardization (2010).
16. Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, et al. Glycaemic index methodology. *Nutr Res Rev.* (2005) 18:145–71. doi: 10.1079/NRR2005100
17. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr.* (1981) 34:362–6. doi: 10.1093/ajcn/34.3.362
18. Altman DG, Royston P. What do we mean by validating a prognostic model? *Stat Med.* (2000) 19:453–73. doi: 10.1002/(SICI)1097-0258(20000229)19:4<453::AID-SIM350>3.0.CO;2-5
19. Bae J. The clinical prediction model for primary care physicians. *Korean J Fam Pract.* (2015) 5:1–6. Available online at: https://www.kjfp.or.kr/journal/view.html?uid=150&vmd=Full

20. Debray TP, Vergouwe Y, Koffijberg H, Nieboer D, Steyerberg EW, Moons KG. A new framework to enhance the interpretation of external validation studies of clinical prediction models. *J Clin Epidemiol.* (2015) 68:279–89. doi: 10.1016/j.jclinepi.2014.06.018
21. Seo SM, Jeong IS. External validation of carbapenem-resistant enterobacteriaceae acquisition risk prediction model in a medium sized hospital. *J Korean Acad Nurs.* (2020) 50:621–30. doi: 10.4040/jkan.20137
22. Lee HS. *Prediction Model of the Glycemic Load by Consuming Mixed Meals: Using Glycemic Index and Glycemic Load of Mixed Meal* (dissertation/master's thesis). Kyung Hee University, Korea (2018).
23. Zimmet PZ, Alberti K, Geroge MM. Introduction: globalization and the non-communicable disease epidemic. *Obesity.* (2006) 14:1. doi: 10.1038/oby.2006.1
24. Park JS, Kim JH, Hyun CS, Joo DH. Analysis of speeding characteristics using data from red light and speed enforcement cameras. *J Korean Soc Transport.* (2016) 34:29–42. doi: 10.7470/jkst.2016.34.1.029
25. Park S, Lim J, Kim H, Lee S. Accidents involving children in school zones study to identify the key influencing factors. *Int J Highway Eng.* (2017) 19:167–74. doi: 10.7855/IJHE.2017.19.2.167
26. Kim HJ, Kim KH. Verification of validity of mpm ii for neurological patients in intensive care units. *J Korean Acad Nurs.* (2011) 41:92–100. doi: 10.4040/jkan.2011.41.1.92
27. Kim DY, Lee H, Choi EY, Lim H. Analysis and evaluation of glycemic indices and glycemic loads of frequently consumed carbohydrate-rich snacks according to variety and cooking method. *J Korean Soc Food Sci Nutr.* (2015) 44:14–23. doi: 10.3746/jkfn.2015.44.1.014
28. Sun L, Ranawana DV, Leow MK, Henry CJ. Effect of chicken, fat and vegetable on glycaemia and insulinaemia to a white rice-based meal in healthy adults. *Eur J Nutr.* (2014) 53:1719–26. doi: 10.1007/s00394-014-0678-z
29. Quek R, Bi X, Henry CJ. Impact of protein-rich meals on glycaemic response of rice. *Br J Nutr.* (2016) 115:1194–201. doi: 10.1017/S0007114515005498
30. Park MH, Nam K, Chung S. Effects of a low glycemic load diet on body weight loss in overweight or obese young adults. *J Nutr Health.* (2020) 53:464–75. doi: 10.4163/jnh.2020.53.5.464

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The Environmental Footprint Associated With the Mediterranean Diet, EAT-Lancet Diet, and the Sustainable Healthy Diet Index: A Population-Based Study

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Providing a growing global population with healthy and sustainable diets is an immediate challenge. In the current study, estimates were obtained for the environmental footprints (land, water, and greenhouse gas (GHG) emissions) in association with the Mediterranean diet (MED) and the EAT-Lancet reference diet, which represents a healthy diet derived from sustainable food systems. We used a newly developed Sustainable Healthy Diet (SHED) index that was validated for the Israeli population by Tepper et al. in 2020.

Methods: A group of 525 participants were recruited via social media, email, and phone. Demographic characteristics, quality of life, and answers to the SHED-index questionnaire were obtained. Dietary assessment was performed using the 116-item Food Frequency Questionnaire (FFQ), which was developed for the Israeli population. Adherence to the MED was calculated using a 9-point score. Adherence to the EAT-Lancet reference diet was assessed through the consumption of 14 food components. The environmental pressure of these dietary patterns was determined based on the "footprint family indicators," which include land, water, and carbon footprints per unit of agricultural and food products. We assigned values for each food comprising the FFQ and calculated the environmental load for each dietary pattern. Statistical analyses were performed using the R package version 4.1.1 to compare environmental footprint values according to tertiles of the MED score, EAT-Lancet score, and SHED score.

Results: The participants ($n = 525$) were 49% women, educated (82% had academic education), and physically active, and only 13% were smokers. The highest tertiles of adherence to the MED, adherence to the EAT-Lancet reference diet, and the SHED index were associated with the lowest GHG emissions and land use, as well as higher water use. Meat consumption contributed the most to land use, while dairy contributed the most to GHG emissions, and fruits contributed the most to water use.

Conclusions: Our analysis reveals that animal protein is the highest contributor to GHG emissions and land use, while fruits and vegetables contribute the most to water consumption. Nevertheless, most of the fruits and vegetables are grown using

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treated wastewater, which reduces environmental pressure. Given these findings, we suggest that MED and EAT-Lancet dietary patterns should be included in national dietary guidelines.

Keywords: sustainable diets, environmental footprints, EAT-Lancet, Mediterranean diet, sustainable food system

INTRODUCTION

The scientific interest in food consumption has evolved in recent decades, given its social and human health implications, direct and indirect pressure on domestic and global environmental systems, and contribution to the wellbeing of individuals and societies (1, 2). A sustainable food system ensures food security and nutrition for all while considering present and future economic, social, and environmental implications (2, 3). Nevertheless, providing a growing global population with healthy and sustainable diets is an immediate challenge (1, 3).

The Mediterranean diet (MED) is well known for its health benefits and has been identified for its environmental benefits as well (4). The diet is characterized by a high intake of plant-based foods; moderate to high intake of fish; moderate to low consumption of poultry, meat, and dairy; high intake of monounsaturated fatty acid (mainly from olive oil); and a moderate amount of wine (1–2 portions per day). As such, the MED's use of natural resources and environmental footprint have been revealed to be low (4, 5). The EAT-Lancet Commission has defined a reference "planetary health diet" based on both sustainability and health. The diet outlines a combination of food groups and ranges of food intake that could optimize human health and the environment (6). In a previous study, the Sustainable and Healthy Diet (SHED) index was developed and validated against both the MED and EAT-Lancet reference diet, which are both considered healthy and sustainable dietary regimens (7).

However, as dietary patterns are shaped by combined social and environmental factors, there is a need to examine not only recommended diets, but also those that are actually practiced, which is critical for advancing healthy and sustainable diets. Few recent studies have examined actual consumption patterns of individuals, households, and societies, their socio-demographic drivers, and their environmental and health implications (4, 5). One of these studies was performed among Italian adults and suggested that the adoption of healthy dietary patterns involves less use of natural resources and greenhouse gas (GHG) emissions (4). Similar results were shown among graduate students in Spain (5).

This paper joins this emerging direction of research in analyzing actual consumption patterns and exploring the gap between recommended diets and actually practiced ones, as well as the implications for the environmental impact of consumption of such diets. To this end, the study explores the differences in the environmental footprint (land, water, and GHGs) of different consumed diets based on local life cycle assessment (LCA) analysis, including unique aspects of the food system in Israel. For this purpose, we created an integrated database within the Food Frequency Questionnaire (FFQ), which includes environmental coefficients for environmental footprints. We analyzed our results with respect to the SHED index (7), the MED (4), and the EAT-Lancet dietary patterns (6) and evaluated the contribution of specific food groups to the environmental footprints using population-based cross-sectional data.

METHODS

Study Population

Using social media, email, and phone calls, we recruited 525 men and women aged 20–66 years during 2018–2020. We approached predefined subpopulations such as vegans and vegetarians; people identifying as secular; rural and urban participants; and individuals with various environmental orientations. Culturally, the population of Israel consists of Jewish populations (the majority) and non-Jewish populations (Muslims, Christians, and Druze), which each have unique dietary consumption patterns. Therefore, the survey includes representation of both Arab and Jewish participants. Using data from the Central Bureau of Statistics in Israel, we aimed to obtain a representative sample of these subpopulations. Once achieving a representative sample for a specific sector, further respondents from this sector were excluded during phone interviews. Participants received the equivalent of 10 USD for completing the questionnaire.

SHED Index

The SHED index is a newly developed, validated index that uses a 30-item questionnaire to assess healthy and sustainable individual diets. The score reflects the nutritional, environmental, and sociocultural aspects of sustainable diets (7). Responses to the items regarding sustainable and healthy eating are recorded on a Likert scale of 1–4. Items are ranked from "Almost never true" to "Almost always true" or from "Never" to "Most of the time." Data on the consumption of beverages and pre-prepared meals are recorded on a scale of six frequencies from "Never" to "Daily." Finally, participants are asked to rate the proportion of plant-based foods in their entire diet on a scale of 0–100%. The questionnaire includes information on demographics, lifestyle, location of food purchases, and frequency of food preparation.

Dietary Assessment

Dietary assessment was performed using the 116-item FFQ, which was developed for the Israeli population. The development and validation process of this questionnaire are described in detail elsewhere (8). The questionnaire is updated annually using a database from the Israeli Ministry of Health (MOH). The MOH data are obtained from the changes and reformulations of food composition and consumption by the Israeli population. For the current study, an updated version from 2018 was used.

Briefly, the FFQ includes 116 food items with nine frequency options ranging from "never or less than once monthly" to "six or more times daily." The questionnaire is semi-quantitative, and a standard portion size is described for each food item. The portion-size estimates are based on information from the MOH. Participants are asked to report their average frequency of consumption during the past year. The questionnaire was self-administered electronically, thus ensuring completeness of the data as a participant could not complete the questionnaire if an item is not answered.

Mediterranean-Diet Score

Adherence to the MED was calculated according to a 9-point score created by Trichopolou et al. (9). For each of the nine components except for alcohol, a value of 0 or 1 is assigned. The units of measurements are serving sizes, and the sex-specific medians of intake of the sample are used as cutoff points. One point is assigned for consumption that is above the median for each of the six protective components (fatty acid ratio, legumes, grains, fruits, vegetables, and fish), and one point is assigned if intake is below the median for the two non-protective components (dairy products and meat). For alcohol, one point is assigned for the mean consumption of 10–50 g/d for men and 5–25 g/d for women.

A score of 9 reflects maximum adherence, indicating that the participant meets all the characteristics of the MED. Based on a sensitivity analysis, we constructed three levels of adherence scores. Low adherence was defined as 0–3 points, medium adherence was 4–6 points, and high adherence was 7–9 points (10).

EAT-Lancet Score

The EAT-Lancet score was calculated based on the calculation created by Kesse-Guyot et al. (11). This calculation is based on the components and cutoff of the EAT-Lancet diet that have been suggested by Willett et al. (6) regarding the consumption of the following 14 food components: whole grains, tubers and starchy vegetables, vegetables, fruits, dairy foods, beef/lamb/pork, poultry, eggs, fish, legumes, nuts, saturated fat, unsaturated fat, and added sugars. The score was computed using the following equation:

$$Eat - Lacent\ score = \frac{\frac{100\times\left\{\sum_{component\ i=1}^{14} a_i\times\left(cut{-}off_i - \frac{consumption_{ij}*2500}{Energy\ consumption_j}\right)\right\}}{cut{-}off_i}}{14}$$

i refers to the 14 food groups, j is the individual participant in the study, $a_i = 1$ for a component to limit, and $a_i = -1$ for a component to promote.

Demographics and Quality of Life

Socio-demographic and lifestyle data included age, sex, employment status, marital status, academic education, area of residence (degree of urbanization), religious identification, crowding (individuals per room), smoking status, level of physical activity, and weight status. Weight status was self-reported as underweight, normal weight, overweight, or obese. Most of these variables were classified as binary variables and reported as percentages. Health-Related Quality of Life (HRQOL) was measured according to the CDC's wellbeing tool and included—"unhealthy days," indicating compromised physical or mental health in the last month, as well as self-rated general health.

Data Collection of Dietary Consumption and Eating Patterns

Survey data were collected using a web application (Qualtrics software, version XM©, Provo, UT, USA. https://www.qualtrics.com). This application reduces missing data. Skipping questions is possible only with pre-definition and was allowed for only items that were decided in advance. The data were extracted in a CSV format and subjected to statistical analysis.

Assigning Environmental Footprints Values

The environmental loads of the studied diets were based on the "footprint family indicators." These indicators have been described as "a set of indicators, characterized by a consumption-based perspective, able to track human pressures on the surrounding environment, where pressure is defined as an appropriation of biological natural resources and CO_2 uptake, emission of GHGs, and consumption and pollution of global freshwater resources" (12). It can be used to identify and assess environmental loads associated with a process, product, or system and allows for examination of potential bio-physical tradeoffs from proposed policy or other measures (12–14). The footprint family indicators of land, water, and carbon footprints per unit of agricultural and food products were analyzed, which required the integration of several kinds of data from different sources. In the following, we describe key data sources and present the calculation procedure for each biophysical indicator.

Land Footprint

The LF included the agricultural land area (m^2) required for growing a unit (kg) of a commodity consumed in Israel. The analysis used data on dozens of crops and processed products from FAOstat (13) to allocate a country's food supply. The allocation procedure first converts processed products and livestock items to primary crop equivalents. Data on commodities grown locally were obtained from FAOstat data, and a bilateral trade matrix was constructed for each crop imported over the last decade. A full description of the data and procedures is available from previous studies (14, 15). Concordance tables and conversion factors used in this analysis are provided as supplementary information.

Water Footprint

The analysis of the water demand for each of the food items included in the research was performed using a database on virtual water (16). The analysis was done using the trade matrix described above to identify domestic and imported food items. The water footprint presented in this research focused on blue water (i.e., irrigation water in cubic meters per ton) in different regions of the world related to the supply of food for consumption in Israel.

TABLE 1 | Characteristics of the study population according to tertiles of the Sustainable and Healthy Eating (SHED) Index score.

		SHED tertiles			
Characteristic	**Overall, $N = 525$[a]**	**Low[a]**	**Medium[a]**	**High[a]**	***p*-value[b]**
Age (years)	32 (10)	30 (8)	32 (10)	35 (10)	<0.001
Gender (women)	259 (49%)	54 (31%)	86 (49%)	119 (68%)	<0.001
Married	259 (49%)	78 (45%)	89 (51%)	92 (52%)	0.4
Employed	396 (76%)	133 (77%)	126 (72%)	137 (78%)	0.4
People per room	0.85 (0.34)	0.88 (0.34)	0.86 (0.37)	0.82 (0.30)	0.2
Education (academic)	432 (82%)	129 (75%)	150 (86%)	153 (87%)	0.004
Smoking	66 (13%)	30 (17%)	21 (12%)	15 (8.5%)	0.043
Physical activity (minutes/week)	161 (155)	127 (152)	169 (155)	186 (152)	<0.001
Self- rated poor health[c]	18 (3.4%)	7 (4.0%)	7 (4.0%)	4 (2.3%)	0.6
Sum of unhealthy days[c]	4.5 (6.0)	4.6 (6.2)	4.7 (6.2)	4.3 (5.6)	0.8
Weight status[d]					0.059
Normal	332 (63%)	98 (57%)	107 (61%)	127 (72%)	
Obese	42 (8.0%)	14 (8.1%)	17 (9.7%)	11 (6.2%)	
Overweight	146 (28%)	58 (34%)	51 (29%)	37 (21%)	
Eating pattern					<0.001
Flexitarian	78 (15%)	9 (5.2%)	22 (12%)	47 (27%)	
High animal-based food	77 (15%)	39 (23%)	21 (12%)	17 (9.7%)	
Omnivore	312 (60%)	121 (70%)	124 (70%)	67 (38%)	
Vegetarian/vegan	56 (11%)	3 (1.7%)	9 (5.1%)	44 (25%)	

[a] *Mean (SD); n (%). SHED tertile values: tertile 1: SHED<17, tertile 2: 17≤SHED <27.9, tertile 3: SHED ≥27.9.*
[b] *Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test.*
[c] *Summary from Health-Related Quality of Life (HRQOL) questions—unhealthy days with compromised physical or mental health in the last month and self-rated general health.*
[d] *Self-reported weight status.*

Carbon Footprint

The CF of each commodity was calculated by incorporating carbon dioxide, nitrous oxide, and methane emissions along the commodity chain for one ton of food consumed in Israel. The results are presented in CO_2 equivalents (CO2e) using factors of 1 kg CO_2/kg CO_2, 310 kg N_2O/kg CO_2, and 21 kg CH_4/kg CO_2 (17). The related emissions integrated two types of data sources. The first followed detailed carbon-footprint review studies, including one by Heller et al. (18), and the second relied on a series of studies based on life-cycle assessments (LCAs) in Israel [e.g., for beef (19), poultry (16), dairy (20), peppers (21), and dates (22)]. In addition, this study also used relevant data for other domestically grown commodities that are now under review and preparation (tomatoes, grapes, avocadoes, and others).

The environmental load values were integrated together into the FFQ to calculate LF, WF, and CF for the questionnaire. Since the survey was designed for nutritional assessment, several nutritionally comparable food items were grouped in the FFQ, along with recipes that were added. In order to assign environmental load values, these groupings and recipes were disaggregated into the original list of 507 food items that originate from the MABAT survey (23). As a result of the integration between the environmental load and dietary intake and quality, each line in the FFQ includes macro and micronutrients and the associated environmental loads of these items.

The total environmental loads for each participant's diet were assessed using the weights of each food item and frequency of consumption. We summed the values of all food items and obtained the impact on the water, land, and GHG emissions of the daily diet of each participant. Although we obtained information for most food items, for a few of them, we did not have available data on their environmental sustainability characteristics. In those cases, we assigned the item the value of the most similar item. However, we did not have enough data for soft drinks, alcoholic drinks, and fish. In addition, data about the LCA for ultra-processed foods were lacking, so they were not included in the current analyses.

Statistical Analysis

All statistical analyses were performed using the R statistical environment (R version 4.1.1), along with a number of libraries that extend the capabilities of the core version of the program. The most significant R libraries used in the analyses were ggplot2, ggpubr, and gtsummary. Dietary intake of food groups and environmental footprints values were represented with the means and standard deviations. Categorical variables were tested using Pearson's Chi-squared test or Fisher's exact test. Fisher was used in cases where there were <5 subjects in one of the groups. Continuous variables were tested with Kruskal-Wallis tests. We classified each participant based on tertiles of the SHED

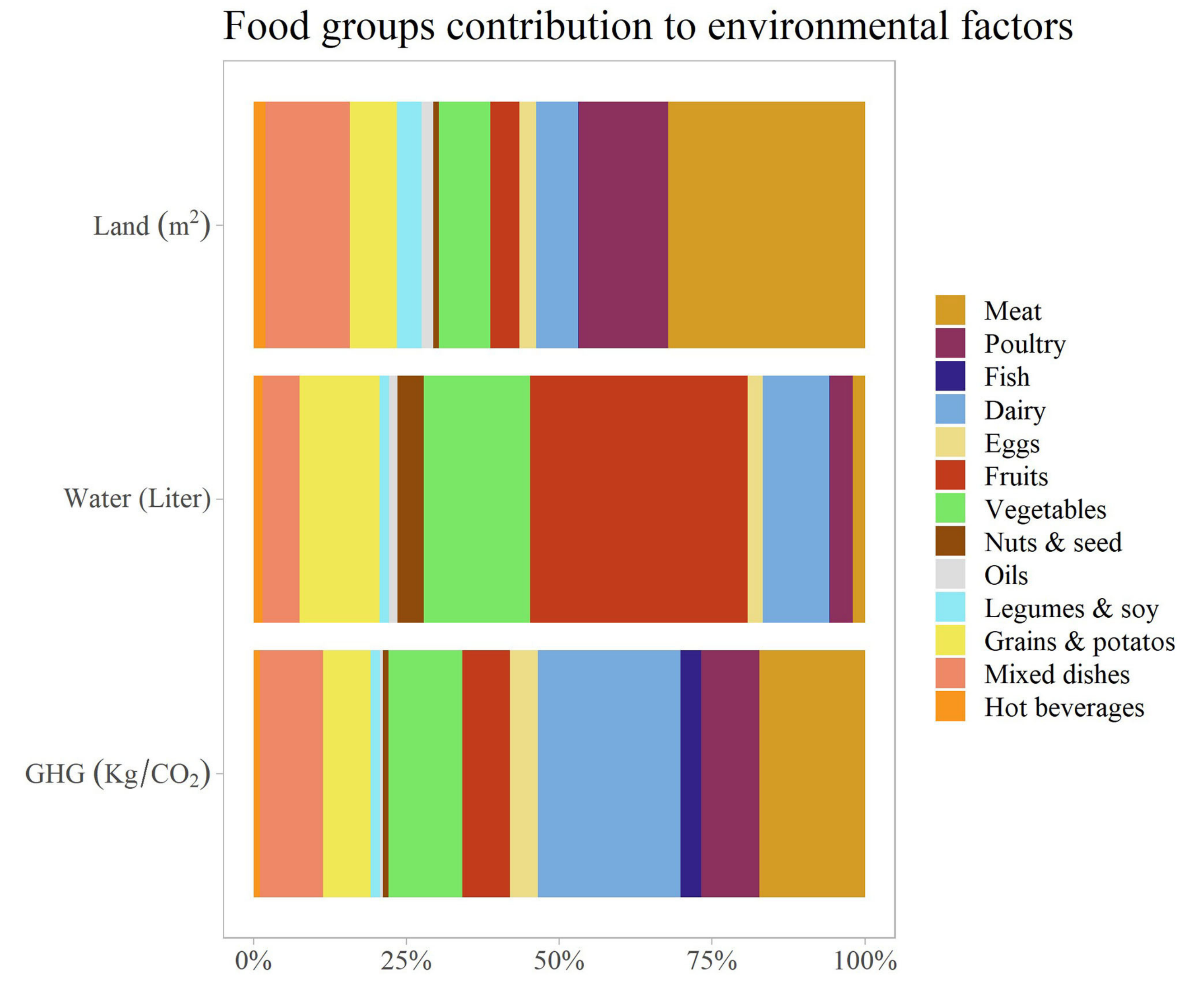

FIGURE 1 | Food groups' relative contributions to land, water, and GHGs footprints.

score, MED score, and EAT-Lancet score (4, 6, 7). Kruskal-Wallis tests were used to assess the differences between the tertiles of each score in regard to GHG emissions, land use, and water use.

RESULTS

The sample included 525 young adult participants aged 20–66 years. The study participants were educated (82% had an academic education) and physically active, and only 13% were smokers. Higher SHED scores were associated with older age, women, higher education, non-smokers, normal weight, flexitarians, and vegetarian/vegans (**Table 1**). The same trends were observed for MED and EAT-Lancet scores (not shown).

The mean footprint use of the total sample was 5.7 ± 3.8 m^2 for land, 422 ± 229 liters for water, and 2.84 ± 1.32 kg/CO_2 for GHGs. The food groups' contributions to the different environmental footprints differ across factors (**Figure 1**). The main contributor to water use was fruits (40%), followed by vegetables (12%) and dairy (11%). The main contributor to land use was meat (30% for beef and 14% for poultry), and the main contributor to GHG emissions was dairy products (26%), followed by meat (14%) and vegetables (14%).

Next, the environmental factors were calculated according to adherence to MED and EAT-Lancet dietary patterns and according to tertiles of the SHED scores (**Figure 2**). Higher adherence to MED was associated with lower land use (high vs. low adherence: 4.07 ± 2.63 vs. 6.6 ± 4.3 m^2, $p < 0.001$), GHG emissions (high vs. low adherence: 2.32 ± 0.9 vs. 3.21 ± 1.32 kg/CO_2, $p < 0.001$), and higher water use (high vs. low adherence: 560 ± 281 vs. 360 ± 203 liters, $p < 0.001$). The same trend was observed for EAT-Lancet adherence and SHED scores (all $p < 0.001$). **Figure 3** shows the mean contribution to the

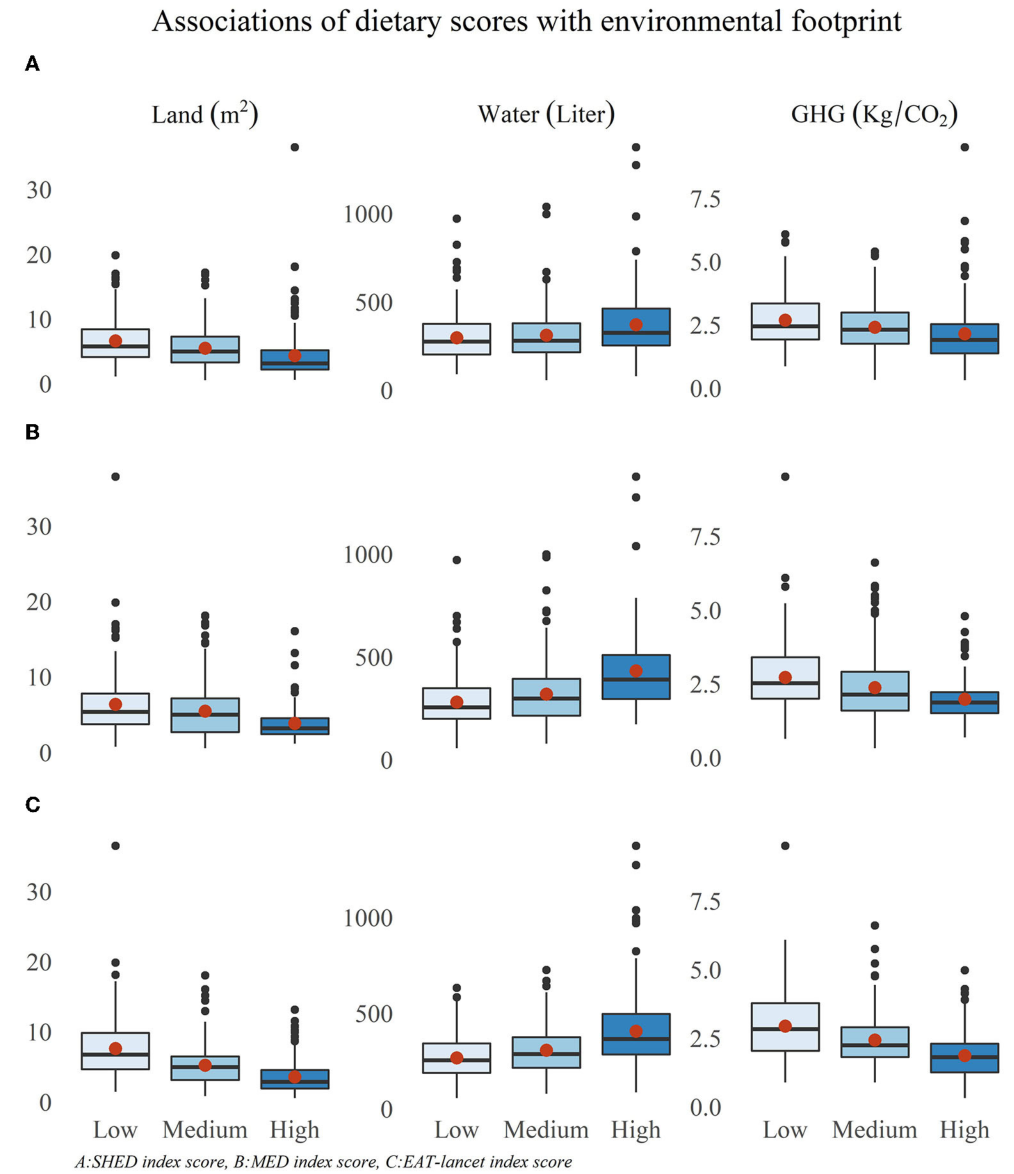

FIGURE 2 | Environmental footprint of different dietary patterns. *All $P < 0.0001$ (Kruskal-Wallis Test).

environmental footprints by food groups for different dietary patterns. At each adherence level of the dietary scores, there was a similar ranking of the food groups to that in **Figure 1**. The main contributor to GHG emissions was dairy products, followed by meat. The main contributor to land use was meat, and the main contributor to water use was fruit intake at each adherence level.

To further explore the relationship between the food groups and the environmental coefficients, we calculated the average consumption of the food-group items for different tertiles of GHG emissions, land use, and water use. **Table 2** shows the mean daily intake of the different food groups by tertiles of environmental footprints. The consumption of plant food groups (fruits, vegetables, nuts/seeds, legumes/soy, and grains/potatoes) did not differ for different land tertiles with the exception of plant oils. The consumption of animal-based food increased for different land tertiles, demonstrating high use of land resources with increased consumption.

The meat intake was 12 times higher in the upper tertile of land use than in the lowest tertile, while poultry intake was 4 times higher, and egg intake was 2.5 times higher. Regarding GHG emissions, only the consumption of nuts/seeds and legumes/soy did not differ between tertiles. In the case of water, only poultry and dairy did not differ between tertiles. These results indicate a narrow range of consumption and smaller influence on water use and GHG emissions.

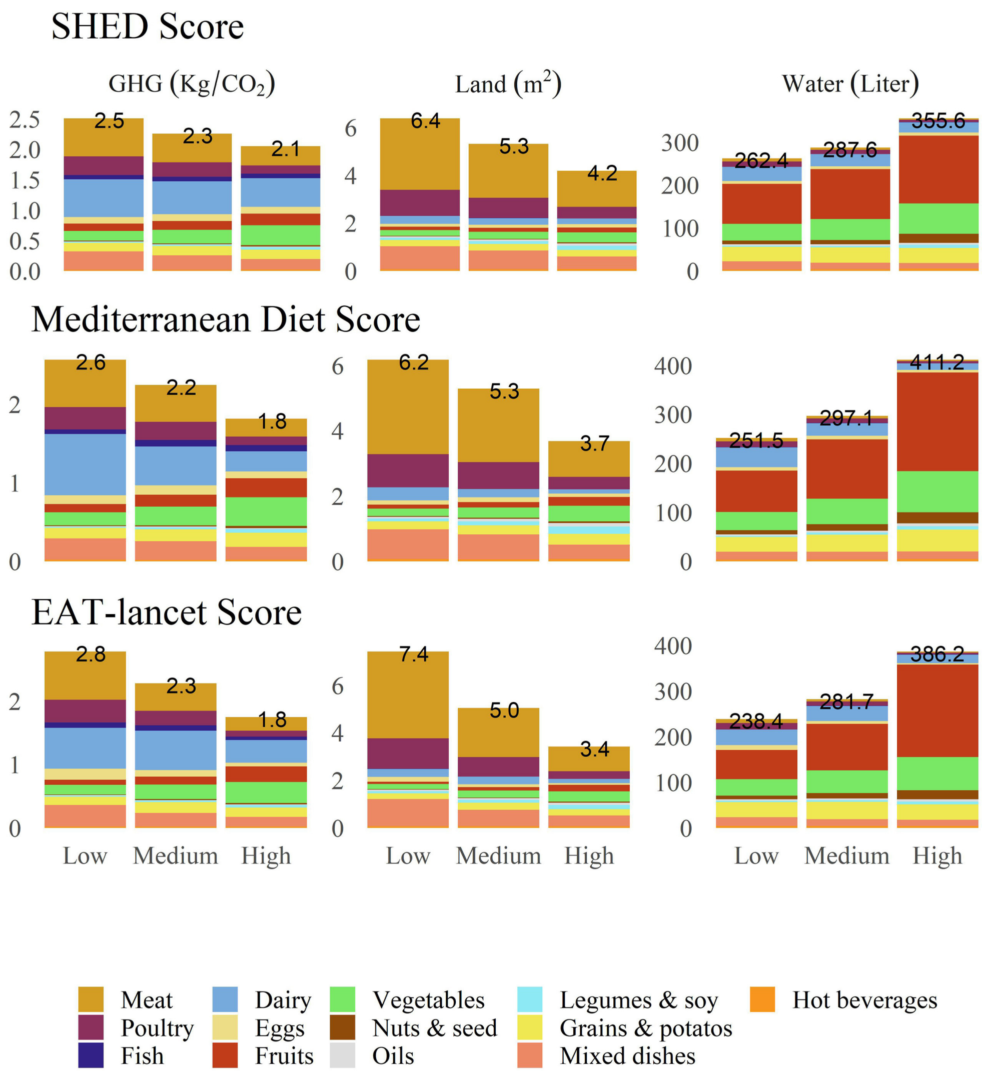

FIGURE 3 | Food groups' contribution to environmental footprints for different dietary patterns. *All $P < 0.0001$ (Kruskal-Wallis Test).

DISCUSSION

The need to assess both human health and environmental footprint of diets has been widely acknowledged. Nevertheless, most studies have focused on theoretical models of dietary guidelines and their potential contributions to sustainability and human health rather than actual consumption (3). The analysis presented in this paper joins a limited number of studies that have identified this gap (4, 5, 24). By analyzing data from a diverse sample of 525 people in Israel, we evaluated real consumption data in regard to adherence to healthy and sustainable diets. These findings are the integrative product of both the studied population's consumption habits and the environmental factors of each studied commodity.

Our findings indicate that the main contributors to water use were fruits, vegetables, and dairy. The main contributors to land use were meat and poultry, and the main contributor to GHG emissions was dairy products. We found that the highest tertiles of adherence to the MED and the EAT-Lancet reference diet (6, 9) were associated with the lowest GHG emissions and land use. On the other hand, the highest tertiles of adherence to the MED and EAT-Lancet were associated with higher water use. To expand our view on sustainability beyond the environmental footprint, adjoining sociocultural, economic, and health aspects we used the SHED index (7). The need to develop methods to include all 4 dimensions of sustainability of the diet (in their case MED) were acknowledged in a recent review by Portugal et al. (24). The results of the SHED index analysis were similar to those obtained for the MED and EAT-Lancet dietary pattern.

The health value of the MED is well established. During the last 20 years MED was shown to benefit health and function, reducing mortality rates (9, 10, 25). The EAT-Lancet

TABLE 2 | Mean daily intake of food groups by tertiles of environmental footprints.

Food Group	Land tertiles				GHG tertiles				Water tertiles				
	Low[a]	Medium[a]	High[a]	p-value[b]	Low[a]	Medium[a]	High[a]	p-value[b]	Low[a]	Medium[a]	High[a]	p-value[b]	Overall, N = 525[a]
Meat	5 ± 6	21 ± 12	61 ± 39	<0.001	9 ± 11	21 ± 16	57 ± 43	<0.001	22 ± 24	28 ± 28	37 ± 44	0.021	29 ± 34
Poultry	32 ± 33	86 ± 59	132 ± 80	<0.001	41 ± 46	78 ± 56	131 ± 82	<0.001	71 ± 56	86 ± 82	94 ± 77	0.062	83 ± 73
Fish	16 ± 20	26 ± 31	27 ± 23	<0.001	15 ± 18	23 ± 20	31 ± 33	<0.001	18 ± 17	24 ± 27	27 ± 30	0.032	23 ± 25
Dairy	323 ± 335	389 ± 339	493 ± 454	<0.001	220 ± 216	386 ± 305	599 ± 485	<0.001	345 ± 300	393 ± 365	468 ± 465	0.086	402 ± 385
Eggs	22 ± 22	34 ± 42	55 ± 76	<0.001	16 ± 15	35 ± 36	60 ± 78	<0.001	24 ± 27	37 ± 44	50 ± 75	<0.001	37 ± 53
Fruits	348 ± 405	323 ± 259	362 ± 349	0.2	273 ± 257	373 ± 381	387 ± 367	<0.001	128 ± 76	274 ± 125	631 ± 445	<0.001	344 ± 343
Vegetables	547 ± 351	558 ± 402	643 ± 556	0.5	471 ± 303	595 ± 408	681 ± 565	<0.001	345 ± 219	555 ± 294	848 ± 580	<0.001	582 ± 446
Nuts seed	11 ± 12	12 ± 15	10 ± 11	0.8	9 ± 11	13 ± 16	11 ± 11	0.3	6 ± 6	10 ± 11	17 ± 17	<0.001	11 ± 13
Oils	4.2 ± 3.6	5.4 ± 5.5	5.8 ± 5.1	0.011	4.2 ± 4.4	5.4 ± 4.8	5.9 ± 5.2	<0.001	3.5 ± 3.2	5.1 ± 4.2	6.9 ± 6.1	<0.001	5.2 ± 4.8
Legumes/soy	84 ± 109	65 ± 62	60 ± 64	0.3	81 ± 108	66 ± 74	62 ± 53	0.5	47 ± 55	66 ± 59	96 ± 111	<0.001	70 ± 82
Grains potatoes	202 ± 131	214 ± 132	230 ± 133	0.074	181 ± 109	219 ± 134	245 ± 144	<0.001	165 ± 83	220 ± 130	261 ± 155	<0.001	215 ± 132
Mixed dishes	104 ± 73	149 ± 86	220 ± 125	<0.001	112 ± 73	149 ± 85	214 ± 131	<0.001	118 ± 78	157 ± 97	199 ± 128	<0.001	158 ± 108
Hot beverages	485 ± 373	499 ± 455	582 ± 489	0.2	458 ± 378	485 ± 423	622 ± 504	0.016	420 ± 364	551 ± 426	595 ± 511	0.004	522 ± 443
Total consumption	3,418 ± 1,084	3,720 ± 1,023	4,283 ± 1,219	<0.001	3,134 ± 943	3,760 ± 927	4,527 ± 1,170	<0.001	2,937 ± 868	3,762 ± 720	4,722 ± 1,103	<0.001	3,807 ± 1,166

[a]*Mean consumption g/day ± SD.*
[b]*Kruskal-Wallis rank sum test.*

as a theoretical dietary pattern targets both health and the environment using evidence based data, indicating that healthy and sustainable diet is achievable (11, 26). In a review by Aleksandrowicz et al. (1), the authors calculated the potential shift in footprint values associated with different dietary patterns. They conclude that shifting from Western diets to more environmentally sustainable dietary patterns can reduce above 70% of GHG emissions and land use, and 50% of water use.

Our findings parallel those of prior studies. In studies from Italy and Spain that used real consumed diet, high adherence to MED pattern was associated with lower GHG emissions and land use (4, 5). The contribution of animal products (meat, poultry, dairy, egg, and fish) constituted the greatest contributor to GHG emissions (19, 21, 22, 27). Higher adherence to the MED or the EAT-Lancet recommended diet was associated with lower GHG emissions in other studies In Spain (the SUN cohort) better adherence to the Spanish MED was associated with decreased environmental pressures in all assessed dimensions including GHG, land and water (5). Likewise, a cross-sectional study among Italian adults showed that omnivorous dietary choices or low adherence to the MED correlated with higher GHG emissions, land, and water use (4, 25). Results in the same direction were found in a non-MED country. Data from the European Prospective Investigation into Cancer and Nutrition—Netherlands (EPIC-NL) (28) cohort showed that the WHO and Dutch dietary guidelines lower the risk of all-cause mortality and moderately lower the environmental impact, while the DASH diet (Dietary Approaches to Stop Hypertension diet), despite leading to similar health outcomes, was associated with higher GHG emissions due to high dairy product consumption in the Netherlands (29). Another study conducted on Italian adults showed that omnivorous dietary choices generated worse carbon, water, and ecological footprints than other plant-based diets, while no differences were found for the environmental impacts of ovo-lacto-vegetarians and vegans (28). Unlike the above studies, in our findings water footprint was higher in the third tertiles of MED, EAT-Lancet and SHED and was connected to fruit intake.

This unique aspect revealed in our analysis needs further discussion. Since fruits and vegetables are more dependent on irrigation than animal-based foods, reducing animal-based foods and increasing plant-based foods do not always correspond with lower water use, as shown by Harris et al. (30). Plant-based foods were major contributors to dietary blue-water footprints. Grosso et al. (4) also found that higher adherence to the MED was not linearly associated with lower water consumption. Higher fruit consumption was also associated with higher water footprints in the United States, particularly the blue-water footprint (17).

However, theoretical dietary models show different results indicating that shifting to a more plant-based diet would reduce the water footprint (31). Other studies also demonstrate that the contribution of fruits and vegetables does not exceed the meat contribution for both blue and green water (32). Indeed, we found that sustainable diets like MED or the EAT-Lancet reference diet are characterized by higher water consumption, but several considerations need to be taken into account. One is that most fruits and vegetables consumed in Israel are grown locally. It follows that given the climatic conditions, most are irrigated, so they would have relatively high rates of blue-water footprints.

Nevertheless, it is important to note that not all blue water is the same as most of the fruit-related water footprint relies on treated wastewater, which reduces environmental pressure (33). Other solutions such as the use of desalinated water and efficient and cost-effective irrigation techniques already exist in Israel, but our findings emphasize the importance of further development of water management, including advanced technologies, reducing water losses, and improving data quality and monitoring for water–food system linkages (32, 33).

Management of the local food systems can also reduce water consumption. There are substantial differences of 2–10-fold in water consumption between different fruit crops (34). Prioritizing certain types of crops that are less burdensome in terms of water requirements while considering their health benefits could be another future direction to increase the health and sustainability of food systems.

As for dairy intake in Israel, the mean intake in our study is 402 ± 385 g per day. This value is higher than the estimated intake in Europe and North America, where the average daily intake is 364.8 g per day according to the PURE study (35). Since most dairy products consumed in Israel are domestically produced, and the production system is based on non-grazing cows, production can occur in a relatively small area (19, 27). In addition, the productivity of Israeli dairy cows is very high, which reduces the footprint per unit of milk (20). Nevertheless, the high demand for dairy products identified in our analysis led to high rates of dairy-related footprints.

According to the EAT-Lancet commission (6) and in accord with the national dietary recommendations, the requirement for different food groups is calculated based on healthy dietary intake within global boundaries. For example, the reference intake is 29 g per day for poultry, 300 g per day for vegetables, 200 g per day for fruits, and 250 g per day for dairy. In our data (**Table 2**), the actual consumption in the lowest tetiles of land GHG and water was nearly similar to the EAT-Lancet recommended diet. The main difference was fruit consumption. Thus, a shift, toward less animal based and more plant-based diets, is beneficial for both health and the environment. The Isocaloric Substitution of Plant-Based and Animal-Based Protein was related with Aging-Related Health Outcomes (36). A recent paper by Eisen and Brown, (37) show that, following a phaseout of livestock production will independently provide persistent drops in atmospheric methane and nitrous oxide levels, and slower carbon dioxide accumulation. This reduction through the end of the century, have the same cumulative effect on the warming potential of the atmosphere as a 25 gigaton per year reduction in anthropogenic CO2 emissions. This level of reduction will provide half of the net emission reductions necessary to limit warming to 2C.

Based on our data, which originate from the FFQ results of 525 participants, it seems that there is no conflict between a healthy and sustainable diet, but there is a need to adjust and optimize dietary patterns in light of recommendations for various populations with different dietary needs. For example, Israel is characterized with mixed Jewish and non-Jewish population, locals and new and established immigrants; and a significant young population alongside a growing share of elderly population. It is important to note that the EAT-Lancet reference diet stems from a theoretical model for a healthy and sustainable diet, whereas our data represent actual dietary patterns of the Israeli population. The results may be a proof of concept that the EAT-Lancet reference diet is indeed feasible.

Strength and Weaknesses

One strength of this study is its ability to assign environmental-footprint values to the FFQ lines. The Israeli FFQ was created based on 24-h recall information that was collected in the Israeli National Health and Nutrition Survey (MABAT) (23). The results of MABAT were available to our group, so we could assign Environmental Footprint values to most of the 570 food items that were on the basic list for the FFQ. The final 116 lines of the FFQ were extracted from the 570 food items. In many cases, when the FFQ is used, the data behind the questionnaire are not available to the researchers. We believe that the use of this basic method results in a more accurate long-term assessment of EF exposure of our participants (8, 38).

Our footprint analysis was based on local supply coefficients. It follows that each analyzed food commodity footprint is considered in terms of whether it was supplied from local sources or imported from several other parts of the world. The footprint was then calculated to reflect the amount of land and water related to the supply from each source (14, 27, 32, 39, 40).

Our study also has several limitations that need to be addressed. One is the use of a convenience sample that was restricted to people who have access to web-based platforms. However, we made an effort to recruit a representative sample including all sectors in Israel. Our sample consists of a high number of educated participants who practice a healthy lifestyle, which may partially limit the generalizability of the results to the general population. While the footprint figures included detailed place-based data and calculations, for some commodities, we had to make some assumptions or exclude some footprint categories. For example, in the case of fish-related footprints, we included only global averages of GHG data and not the other footprint coefficients.

CONCLUSION

Our analysis of consumed diets revealed that animal protein is associated with the highest GHG emissions and land use, while fruits and vegetables were associated with the highest water consumption. Nevertheless, most of them are grown using treated wastewater, which reduces environmental pressure. The differences in water consumption for different fruit crops support the need to prioritize certain types of crops, which should be less burdensome in terms of water requirements while considering their health benefits. Given these findings, we suggest that adherence to MED and EAT-Lancet dietary patterns should be included in national dietary guidelines and encouraged for consumption by all. Furthermore, our data could be used as a database to create healthy and sustainable diet recommendations while adjusting for nutritional needs and health status, as well as maintaining diversity within dietary patterns.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tel-Hai College (#09/2017-2). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ST planned and conducted the research and wrote the first draft of the manuscript. MK calculated the environmental footprints and co-authored the manuscript. DS created the combined database and co-authored the manuscript. KA analyzed the data and co-authored it. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Aleksandrowicz L, Green R, Joy EJ, Smith P, Haines A. The impacts of dietary change on greenhouse gas emissions, land use, water use, and health: a systematic review. *PLoS ONE.* (2016) 11:e0165797. doi: 10.1371/journal.pone.0165797
2. Mason P, Lang T. *Sustainable Diets: How Ecological Nutrition Can Transform Consumption and the Food System. Abingdon:* Routledge (2017).
3. Conijn JG, Bindraban PS, Schröder JJ, Jongschaap R. Can our global food system meet food demand within planetary boundaries? *Agric Ecosyst Environ.* (2018) 251:244–56. doi: 10.1016/j.agee.2017.06.001
4. Grosso G, Fresán U, Bes-Rastrollo M, Marventano S, Galvano F. Environmental impact of dietary choices: role of the mediterranean and other dietary patterns in an italian cohort. *Int J Environ Res Public Health.* (2020) 17:1468. doi: 10.3390/ijerph17051468
5. Fresán U, Martínez-Gonzalez M, Sabaté J, Bes-Rastrollo M. The Mediterranean diet, an environmentally friendly option: evidence from the Seguimiento Universidad de Navarra (SUN) cohort. *Public Health Nutr.* (2018) 21:1573–82. doi: 10.1017/S1368980017003986
6. Willett W, Rockstrom J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4
7. Tepper S, Geva D, Shahar DR, Shepon A, Mendelsohn O, Golan M, et al. The SHED index: a tool for assessing a sustainable HEalthy diet. *Eur J Nutr*. (2021) 60:3897–909. doi: 10.1007/s00394-021-02554-8
8. Shai I, Rosner BA, Shahar DR, Vardi H, Azrad AB, Kanfi A, et al. Dietary evaluation and attenuation of relative risk: multiple comparisons between blood and urinary biomarkers, food frequency, and 24-hour recall questionnaires: the DEARR study. *J Nutr.* (2005) 135:573. doi: 10.1093/jn/135.3.573
9. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med.* (2003) 348:2599–608. doi: 10.1056/NEJMoa025039
10. Tepper S, Alter Sivashensky A, Rivkah Shahar D, Geva D, Cukierman-Yaffe T. The association between Mediterranean diet and the risk of falls and physical function indices in older type 2 diabetic people varies by age. *Nutrients.* (2018) 10:767. doi: 10.3390/nu10060767
11. Kesse-Guyot E, Rebouillat P, Brunin J, Langevin B, Allès B, Touvier M, et al. Environmental and nutritional analysis of the EAT-Lancet diet at the individual level: insights from the NutriNet-Santé study. *J Clean Prod.* (2021) 296:126555. doi: 10.1016/j.jclepro.2021.126555
12. Galli A, Wiedmann T, Ercin E, Knoblauch D, Ewing B, Giljum S. Integrating ecological, carbon and water footprint into a "footprint family" of indicators: definition and role in tracking human pressure on the planet. *Ecol Ind.* (2012) 16:100–12. doi: 10.1016/j.ecolind.2011.06.017
13. FAOstat. *Food and Agricultural organization Statistics*. (2020). Avilable online at: https://www.fao.org/faostat/en/#home (accessed September, 2021).
14. Fridman D, Kissinger M. An integrated biophysical and ecosystem approach as a base for ecosystem services analysis across regions. *Ecosystem Services.* (2018) 31:242–54. doi: 10.1016/j.ecoser.2018.01.005
15. Kastner T, Erb K, Haberl H. Rapid growth in agricultural trade: effects on global area efficiency and the role of management. *Environ Res Lett.* (2014) 9:034015. doi: 10.1088/1748-9326/9/3/034015
16. Mekonnen MM, Hoekstra AY. A global assessment of the water footprintof farm animal products. *Ecosystems*. (2012) 15:401–15. doi: 10.1007/s10021-011-9517-8
17. Tom MS, Fischbeck PS, Hendrickson CT. Energy use, blue water footprint, and greenhouse gas emissions for current food consumption patterns and dietary recommendations in the US. *Environ Syst Decis.* (2016) 36:92–103. doi: 10.1007/s10669-015-9577-y
18. Heller MC, Keoleian GA, Willett WC. Toward a life cycle-based, diet-level framework for food environmental impact and nutritional quality assessment: a critical review. *Environ Sci Technol.* (2013) 47:12632–47. doi: 10.1021/es4025113
19. Kissinger M, Dickler S. Interregional bio-physical connections—a 'footprint family'analysis of Israel's beef supply system. *Ecol Ind.* (2016) 69:882–91. doi: 10.1016/j.ecolind.2016.05.024
20. Triky S, Kissinger M. An integrated analysis of dairy farming: direct and indirect environmental interactions in challenging bio-physical conditions. *Agriculture*. (2022) 12:480.
21. Ravits-Wyngaard S, Kissinger M. *Embracing a Footprint Assessment Approach for Analyzing Desert Based Agricultural System: The Case of Medjool Dates*. Nekudat Chen: Yad Hanadiv (2020).
22. Wyngaard SR, Kissinger M. Materials flow analysis of a desert food production system: the case of bell peppers. *J Clean Prod.* (2019) 227:512–21. doi: 10.1016/j.jclepro.2019.04.139
23. Peng W, Goldsmith R, Shimony T, Berry EM, Sinai T. Trends in the adherence to the Mediterranean diet in Israeli adolescents: results from two national health and nutrition surveys, 2003 and 2016. *Eur J Nutr.* (2021) 60:3625–38. doi: 10.1007/s00394-021-02522-2
24. Portugal-Nunes C, Nunes FM, Fraga I, Saraiva C, Gonçalves C. Assessment of the methodology that is used to determine the nutritional sustainability of the mediterranean diet—a scoping review. *Front Nutr.* (2021) 8:7722133. doi: 10.3389/fnut.2021.772133

25. Gantenbein KV, Kanaka-Gantenbein C. Mediterranean diet as an antioxidant: the impact on metabolic health and overall wellbeing. *Nutrients.* (2021) 13:1951. doi: 10.3390/nu13061951
26. Lassen AD, Christensen LM, Trolle E. Development of a Danish adapted healthy plant-based diet based on the eat-lancet reference diet. *Nutrients.* (2020) 12:738. doi: 10.3390/nu12030738
27. Triky S, Kissinger M. An integrated analysis of dairy farming: direct and indirect environmental interactions in challenging bio-physical conditions. *Agriculture.* (2022) 12:1–11. doi: 10.3390/agriculture12040480
28. Rosi A, Mena P, Pellegrini N, Turroni S, Neviani E, Ferrocino I, et al. Environmental impact of omnivorous, ovo-lacto-vegetarian, and vegan diet. *Sci Rep.* (2017) 7:1–9. doi: 10.1038/s41598-017-06466-8
29. Biesbroek S, Verschuren WM, Boer JM. van de Kamp, Mirjam E, Van Der Schouw, et al. Does a better adherence to dietary guidelines reduce mortality risk and environmental impact in the Dutch sub-cohort of the European Prospective Investigation into Cancer and Nutrition? *Br J Nutr.* (2017) 118:69–80. doi: 10.1017/S0007114517001878
30. Harris F, Moss C, Joy EJ, Quinn R, Scheelbeek PF, Dangour AD, et al. The water footprint of diets: a global systematic review and meta-analysis. *Advances in Nutrition.* (2020) 11:375–86. doi: 10.1093/advances/nmz091
31. Tompa O, Lakner Z, Oláh J, Popp J, Kiss A. Is the sustainable choice a healthy choice? —Water footprint consequence of changing dietary patterns. *Nutrients.* (2020) 12:2578. doi: 10.3390/nu12092578
32. Fridman D, Biran N, Kissinger M. Beyond blue: an extended framework of blue water footprint accounting. *Sci Total Environ.* (2021) 777:146010. doi: 10.1016/j.scitotenv.2021.146010
33. Ringler C, Agbonlahor M, Baye K, Barron J, Hafeez M, Lundqvist J, et al. Water for food systems and nutrition. *Food Syst Summit Brief.* (2021) 1–13. doi: 10.48565/scfss2021-tg56
34. Mekonnen MM, Hoekstra AY. The green, blue and grey water footprint of crops and derived crop products. *Hydrol Earth Syst Sci.* (2011) 15:1577–600. doi: 10.5194/hess-15-1577-2011
35. Dehghan M, Mente A, Rangarajan S, Sheridan P, Mohan V, Iqbal R, et al. Association of dairy intake with cardiovascular disease and mortality in 21 countries from five continents (PURE): a prospective cohort study. *Lancet.* (2018) 392:2288–97. doi: 10.1016/S0140-6736(18)31812-9
36. Zheng J, Zhu T, Yang G, Zhao L, Li F, Park Y, et al. The Isocaloric substitution of plant-based and animal-based protein in relation to aging-related health outcomes: a systematic review. *Nutrients.* (2022) 14:272. doi: 10.3390/nu14020272
37. Eisen MB, Brown PO. Rapid global phaseout of animal agriculture has the potential to stabilize greenhouse gas levels for 30 years and offset 68 percent of CO2 emissions this century. *PLoS Climate.* (2022) 1:e0000010. doi: 10.1371/journal.pclm.0000010
38. Shahar D, Shai I, Vardi H, Brener-Azrad A, Fraser D. Development of a semi-quantitative Food Frequency Questionnaire (FFQ) to assess dietary intake of multiethnic populations. *Eur J Epidemiol.* (2003) 18:855–61. doi: 10.1023/A:1025634020718
39. Kissinger M. Approaches for calculating a nation's food ecological footprint—the case of Canada. *Ecol Ind.* (2013) 24:366–74. doi: 10.1016/j.ecolind.2012.06.023
40. Kissinger M, Sussmann C, Dorward C, Mullinix K. Local or global: a biophysical analysis of a regional food system. *Renew Agric Food Syst.* (2019) 34:523–33. doi: 10.1017/S1742170518000078

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Practical Implementation of the BLW Method During the Expansion of the Infant Diet—A Study Among Polish Children

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The aim of the study was to verify the knowledge of mothers of children under 3 years of age about the Baby Led Weaning (BLW) feeding model and their practical implementation of this method. The study involved 761 mothers and their children. After analysis of the inclusion and exclusion criterion, the information provided by women 699 aged 21–48 years was included in the final data analysis. In the study group, most children were breastfed for 6 months to 1 year ($n = 256$, 36.7%), 1 year to 2 years ($n = 179$, 25.6%) and over 2 years ($n = 71$, 10.2%). Starting dietary expansion before 17 weeks of age was implemented in 47 (6.7%) children, between and 17–26 weeks of age in 328 (46.9%) children, and after 26 weeks of age in 324 (46.3%) children. Feeding food and dishes from the family table was practiced by 518 (74.1%) mothers. Spoon-feeding was practiced by 529 (75.6%) children, 157 (22.4%) children were fed this way sometimes. Taking into account the above data, feeding with the BLW method was used in 170 children (24.2%). In the examined group of mothers the use of the BLW method in feeding their children, especially during diet expansion, was declared by 408 women (74.8%). The child's independent decision concerning what the child will eat and what is according to the BLW method is accepted by 434 (62.1%) mothers. Among the positive aspects of using the BLW method, the women surveyed indicated the child's independence, while among the disadvantages, the omnipresent mess and chaos when eating meals.

Keywords: Child Nutrition, Baby Led Weaning (BLW), expanding the diet of infants, complementary feeding, child diet

INTRODUCTION

In the postnatal period, and especially in the first 33 months of a child's life, nutrition is a form of synbiotic directed at the gastrointestinal tract, more specifically at the intestinal microbiota, with a regulatory effect on the gut-brain axis. Because of this issue, it is important to shape pro-healthy eating habits in children (1). The recommended way of diet expansion, which at the same time introduces a lot of controversies, is the *Baby Led Weaning* (BLW) feeding model (2, 3). The Polish

Society of Gastroenterology, Hepatology and Child Nutrition (PTGHiZD) defines this method of feeding as feeding with the omission of mushy consistency served with cutlery, with the exclusive implementation of solid consistency eaten by an infant on its own (4). It is a method of expanding and supplementing feeding between 17 and 26 weeks of age (4). According to the World Health Organization (WHO), a baby should be breastfed exclusively with breast milk for the first 6 months. After the introduction of complementary foods, WHO and ESPGHAN recommend continuing breastfeeding for as long as desired by mother and child (5, 6). WHO recommends starting the introduction of complementary foods around the sixth month of a child's life (6). On the other hand, ESPGHAN and PTGHiZD recommend starting the introduction of solid foods after the 17th and no later than the 26th week of a child's life. These two different positions may confuse parents as to when they should start expanding the child's diet (4, 5). The introduction of new products to the infant's diet also aims to prepare the infant for a more varied diet later in life, including learning tastes and textures (7, 8). In most infants, between 17 and 26 weeks of age, the ability to accept solid foods matures. It is not until the infant is 17 weeks old that the gastrointestinal tract and kidneys are mature enough for the infant to assimilate non-dairy foods. The swallowing reflex and the ability to accept puréed food from a spoon appear between 4 and 6 months of age. The introduction of foods before the age of 3–4 months also carries a risk of allergy (5). Infants between 17 and 26 weeks of age develop the ability to sit with support, acquire neuromuscular maturity that allows them to control head and neck movements, and to eat from a spoon. At this time, the reflex to remove foreign bodies from the mouth, which made feeding with food other than liquid difficult, ceases (4–6). All these aspects allow for the introduction of feeding with the BLW method. Numerous parents' doubts about its use, not based on scientific reports (Evidence-Based Medicine—EBM), focus on the fear of nutritional deficiencies (especially of microelements, e.g., iron, zinc, vitamin B_{12}), growth retardation, disorders of psychomotor development, and choking defined as a life-threatening condition involving partial or complete obstruction of the upper airways. Often their concerns also relate to wasting food, as well as the appearance of the environment after the child has eaten a meal on their own (2, 4). In the BLW method, eating together as a family and at meal times is important. This approach can give the infant more control over food intake. This can lead to better eating patterns and reduce the risk of overweight and obesity. Given the self-selection of parents and infants who currently use the BLW method, and the limited observational data available however, it is not possible to draw conclusions. Furthermore, there is a lack of data on whether infants who are traditionally spoon-fed receive sufficient nutrients, including energy and iron, or whether they consume more or they consume a greater variety of foods. ESPHGAN indicates that these issues should be investigated in RCTs. A modified version of BLW called BLISS—emphasizes the importance of introducing iron- and energy-rich energy-rich complementary foods and the avoidance of foods that may present choking hazards. A small observational pilot study suggests that this approach had some benefit in increasing iron-rich foods consumed by infants (4, 5). In a study by Neves et al. (9) note that there is still insufficient scientific and quality evidence to confirm that the BLW method is the most appropriate form of feeding into the diet of infants. Health professionals from New Zealand and Canada were concerned about the potential for energy and iron deficiencies in BLW children, which can cause disruption to children's growth and normal development (9–11).

TABLE 1 | Proposal of implementation of complementary products.

6–7 months of the child's life	8–12 months of the child's life
• Soft vegetable pieces in cooked form (potato, carrot, courgette, pumpkin, beetroot, cauliflower, broccoli)	• Meals of a lumpy consistency
• Puree of the above vegetables • Pureed fruit (peach, apricot, pear, banana, apple)	• Food served in pieces in the child's hand
• Groats from gluten cereals and gluten-free cereals in thick consistency • Protein in the form of meat—poultry, fish • Vegetable oils, groundnuts, grains, seeds • Egg yolk • Fermented milk products (buttermilk, kefir, natural yogurt) • Mineral water, still in unlimited quantity	• The process of continuing the model of expanding nutrition in the form of new foods from the group of products such as fruit, vegetables, groats, pasta, bread, egg white, nuts, etc.

Own elaboration based on pediatric Dietetics, chapter 2.5 Expanding the infant's diet in practical aspects. Medical University of Silesia Publishers, 2020 (12).

In response to these concerns, suggestions for safe products adapted to the age of the child can be made, based on the experience of a specialist in clinical and pediatric dietetics (12) (**Table 1**).

Another method aimed at partially alleviating parents' anxiety is *Baby-Led Introduction to Solids* (BLISS). This modification consists in introducing products adapted to the child's developed body motor mechanism, followed by biting, chewing and swallowing, and fortification in the presence of iron. When implementing both dietary models, it is important to keep in mind the sensory sensitivity of the child without leading to the appearance of aversion (13, 14). The sensory diversity of children is an important issue, so three criteria referring to the level of inclination toward the child's food preferences should be taken into account (15) (**Table 2**). BLISS resources are consistent with the BLW philosophy but address three key concerns that some health professionals have expressed about BLW: inadequate iron intake, choking and stunted growth. By: checking foods before serving to make sure they are soft enough, avoiding foods that form a crumb in the mouth, foods should be at least as long as the baby's fist, at least one side of the food, the baby's sitting position so they sit up straight while eating—never leaning back, that an adult is always with the baby, and that the baby does not put whole foods in their mouth—the baby needs to do this at their own pace and under their own control (16).

TABLE 2 | Examples of sensory diversity among children.

The tendency to the child's food preferences	(1) Food habituation to milk consumption with a concomitant desire to consume introduced new products (vegetables, fruit, eggs, porridge, meat) occurs in breastfed and artificially mixed children
	(2) Eating habits focusing exclusively on milk consumption through breastfeeding and the introduction of artificial mixtures—modified milk, with aversion to new complementary products
	(3) Food habits focusing on food aversion toward milk in the form of a bottle and breastfed, but sensory inclination toward complementary products initially in thick form served with a spoon (porridge, soups, fruit), then in solid form (pieces of products).

Own elaboration based on what do happy toddlers eat? Nutritional Publishers, 2020 (15).

The key influence on the positive effects of their application and the introduction of complementary foods is the clinical condition of the infant. Simultaneously occurring symptoms of food neophobia or food selectivity and the period of product administration (preferably after 6 months of age), the acceptance of known foods (facilitates the introduction of new products), the method of breastfeeding (brings many benefits), as well as the prenatal period in the past influence the way of expanding the diet of a small child. According to the concept of metabolic programming, already the diet of a pregnant woman shows an influence on the composition of amniotic fluid, and this, in time, will influence the way of diet expansion in an infant and his/her willingness to learn new tastes (17–20). The use of dietary expansion methods in the form of BLW or BLISS predisposes to the appearance of elements of dietoprophylaxis and health prevention in the later stages of a child's adolescence. They include low risk of metabolic diseases (overweight, obesity, hybrid diabetes), correct regulation of the hunger and satiety center—control of physiological hunger, developed on a differentiated, rational level eating habits by regulating the food given by the infant independently. Therefore, it is the toddler who will decide on the number of portions in conditions devoid of emotional pressure by the parents, with all the hygienic and safety rules monitored by them, maintained during the meal. It should be mentioned that among the pro-health elements there is also the education of parents and the use of the family table method. Next, it seems important to shape motor skills among infants and an independent relationship with food, as well as to stimulate the child's masticatory system (21–23).

The aim of the study was to verify the knowledge of the Baby Led Weaning feeding model among mothers of children up to 3 years of age and their practical implementation of this method.

MATERIALS AND METHODS

Test Group

An exploratory cross-sectional study was conducted from April to June 2021 among randomly selected 761 mothers and their children, aged up to 3 years, residing in Upper Silesia. Mothers were recruited at randomly selected nurseries and pediatric clinics during mandatory vaccination visits. A list of public and private nurseries has been compiled, as well as a list of pediatric clinics where vaccinations are carried out for children aged 0–3 years—these establishments are both public and non-public and have a contract with the Narodowy Fundusz Zdrowia (National Health Fund), which finances vaccinations in Poland. We have defined randomization nurseries and pediatric outpatient clinics. 10 nurseries and 10 pediatric outpatient clinics were drawn from the list. In each nursery and pediatric clinic 40 parents of children aged 0–3 years were invited to participate in the study. All participants were informed about the purpose of the study, voluntary participation in the study, and anonymity, and were asked to accept the data sharing policy. After considering the inclusion and exclusion criteria, information collected from 699 women was included in the final analysis.

Due to the nature of the study, consent was sought from the Bioethics Committee of the Medical University of Silesia in Katowice. By the Resolution No. KNW/0022/KB1/151/I/11 the Bioethics Committee issued a positive opinion on the project. The study is in accordance with the Declaration of Helsinki.

Rationale for the Selection of the Group

According to the current Polish law, after giving birth a mother is entitled to a maternity leave of 20 weeks for one child, 31 weeks for twins, 33 weeks for three children, 35 weeks for four children, 37 weeks for five and more children (24). After this time, parental leave can be taken, which lasts 32 weeks in the case of the birth of one child and entitles both parents to take it. Reports from the Social Insurance Institution indicate that in Poland, from January to May 2021, more than 246,000 parents, including only 1,900 men, benefited from maternity benefits for the period of parental leave (25). Therefore, mothers were invited to the study on infant diet expansion, as they are the ones who mostly spend time with their children and are responsible for expanding their diet.

Inclusion and Exclusion Criteria

Inclusion criteria were: female sex, having a child aged 0–36 months (up to 3 years) of age, consenting to the study, and completing the questionnaire correctly and completely.

On the other hand, the criteria for exclusion from the study were: lack of consent to participate in the study, incorrectly completed questionnaire, including non-response to questions, and the child's age above 36 months.

Research Tool

The research tool was a survey questionnaire, which consisted of several parts. The survey was conducted personally by qualified interviewers who were thoroughly trained on how to fill in the questionnaires—they were students of Dietetics at the Medical University of Silesia in Katowice. The questionnaires were filled in and entered by the interviewers in electronic form using tablets. The first part was a metric that asked women about their age, place of residence, education, height, and body weight. In the study conducted at the pediatric outpatient clinic, all children had their weight and length/height measured by nurses.

In the nursery, on the other hand, weight and length/height measurements were taken by staff employed in the institution. Mothers, on the other hand, entered their own weight and height. There were also questions about the current work situation, education, marital status, number of children, and age of the youngest child.

The next part of the questionnaire concerned the basic knowledge about expanding the diet of a child and expanding the diet of a child using the BLW method. The part of the questionnaire concerning the method of extending the diet of a child included questions about the time of starting and the method of extending the diet of a child, the use of the BLW method, and indicating the advantages and disadvantages of this method or risks in independent eating by a child. At the same time, the questionnaire included questions about giving food to the child from the family table, feeding the child with a spoon, allowing the child to decide independently what and how much to eat, the order of introducing particular food products to the diet, the consistency of meals from which the expansion of the child's diet should begin, the type of thermal processing used to prepare meals, the time when the child received sweets for the first time.

The questionnaire was based on current dietary recommendations for the group of the youngest children and the method of dietary expansion developed by PTGHiZD (4). A pilot study was conducted on a group of 50 mothers in order to validate the questionnaire and to check the relevance and acceptability of the questions included in it. Reproducibility of responses was tested by comparing responses in the same group of subjects. Pilot study 2 took place 1 month after the pilot study to avoid freshness effects. To assess the reproducibility of the results obtained with the questionnaire used, the x parameter (Kappa) was calculated for each question in the questionnaire (results obtained in the pilot study and pilot study 2). For 67.3% of the questions, a very good ($x \geq 0.80$) concordance of responses was obtained, and for 28.4% of the questions a good ($0.79 \geq x \geq 0.60$) concordance of methods was obtained. For only 4.3% of the questions in the questionnaire analyzed, the concordance between the results obtained at baseline and in the repeat survey was moderate ($x < 0.59$).

Also analyzed, Cronbach's α coefficient for the normalization sample was 0.87, which indicates high reliability and repeatability of the questionnaire. The pilot study allowed us to validate the questions included in the questionnaire. Cronbach's alpha for the relevant part of the study was estimated at 0.86.

Statistical Analysis

Statistical analysis was performed using the software in Statistica v. 13.1 (StatSoft Inc., Tulsa, OK, USA). Non-parametric tests were used for statistical elaboration. Differences between groups were tested by Pearson's Chi-square test, with Fisher's exact, Yates, and the level of statistical significance was taken at $p < 0.05$.

TABLE 3 | Characteristics of the study group of mothers.

	n	%
Age of mothers		
21–25 years	18	2.6
26–30 years	153	21.8
31–35 years	261	37.4
36–40 years	227	32.5
Over 40 years	40	5.7
Place of residence of mothers		
City over 500 thousand inhabitants	644	92.1
City with 100–500 thousand inhabitants	26	3.7
City with 20–100 thousand inhabitants	14	2.0
Village of	15	2.2
The educational level of mothers		
Higher	613	87.8
Averages	71	10.2
Professional	9	1.3
Basic	5	0,7
Marital status of mothers		
Married	559	80.1
Miss	97	13.9
Informal relationship	25	3.6
Divorced	16	2.3
Widow	1	0.1
Maternal body weight by BMI		
Underweight	29	4.2
Correct body weight	459	65.7
Overweight	136	19.5
Obesity	52	7.4
No data available	22	3.2
Number of children		
One	250	35.8
Two	331	47.4
Three or more	117	16.8

RESULTS

Characteristics of the Study Group of Mothers and Their Children

The characteristics of the study group of mothers are shown in **Table 3**. The largest group were mothers aged 31–35 years ($n = 261$; 37.4%), with a mean age of 33.9 ± 4.7 years. In the group of surveyed mothers, women living in a city with more than 500 000 inhabitants were $n = 644$; 92.1%. Higher education among the mothers studied was $n = 613$; 87.8%. On the other hand, marital status—married had $n = 559$; 80.1%. Analyzing body weight composition, normal body weight was $n = 459$; 65.7%. Most mothers in the study group had two children ($n = 331$; 47.4%).

The characteristics of the studied group of children and the diet of this group are presented in **Table 4**. The largest group were children aged 2–2.5 years ($n = 299$, 42.7%), followed by 1–1.5 years ($n = 168$, 24.0%) and 2.5–3 years ($n = 118$, 16.9%). Most children were fed breast milk for 6 months to 1 year (n

TABLE 4 | Characteristics of the studied group of children and their diet.

	n	%
Age of child		
0–0.5 years of age	38	5.4
0.5–1 year old	32	4.5
1–1.5 years old	168	24.0
1.5–2 years	44	6.3
2–2.5 years	299	42.7
2.5–3 years	118	16.9
Length of breastfeeding by mothers		
Less than a month	42	6
1–2 months	46	6.6
3–4 months	56	8
5–6 months	47	6.7
6 months to 1 year	256	36.7
1 to 2 years	179	25.6
Over 2 years	71	10.2
Time to start expanding the diet		
Before week 17	47	6.7
Between 17 and 26 weeks	328	46.9
After 26 weeks	324	46.3
Serving of products/dishes from the family table		
Yes	518	74.1
Sometimes	153	21.9
Not	28	4.0
Feeding the baby with a spoon		
Yes	529	75.6
Sometimes	157	22.4
Not	13	1.8

= 256, 36.7%), followed by 1–2 years (n = 179, 25.6%). Dietary expansion before 17 weeks of age was implemented in 47 (6.7%) children, between 17 and 26 weeks of age in 328 (46.9%) children (ESPGHAN and PTGHiZD recomendation) and after 26 weeks of age in 324 (46.3%) children. Feeding food and dishes from the family table was practiced by 518 (74.1%) mothers. Spoon-feeding was practiced by 529 (75.6%) children and 157 (22.4%) were occasionally fed in this way. Considering the above data, BLW feeding was used in 170 children (24.2%).

Knowledge and Methods to Expand the Child's Diet

In the part of the study concerning the knowledge of dietary expansion with the BLW method 545 (78.0%) women recognized this method of dietary expansion and demonstrated its knowledge. The main source of knowledge on this subject for the participating women was the Internet (n = 244; 43, 96%), followed by friends (n = 117; 21.8%), books (n = 75; 13.51%), internet forums for mothers and professional literature, respectively (n = 35; 6.31%) and (n = 34; 6.13%). Respectively, 14 (2.52%) and 13 (2.34%) mothers received information on the BLW method from their doctor or in the birthing school

TABLE 5 | Mothers' nutritional knowledge of BLW.

	n	%
Knowledge of the BLW method as a means of dietary expansion		
Yes	545	78.0
Not	154	22.0
Source of information about the BLW method		
Internet	244	43.96
From friends	117	21.08
From books	75	13.51
From a forum for mothers	35	6.31
From professional literature (e.g., Pubmed)	34	6.13
Other sources of knowledge	15	2.70
From the doctor	14	2.52
From the birthing school	13	2.34
From the nutritionist	4	0.72
From the midwife	3	0.54

(**Table 5**). Of the 545 mothers who declared knowledge of the BLW method, 480 (88.1%) considered this method as appropriate.

The use of the BLW method in feeding their children, especially during diet expansion, was declared by 408 (74.8%) women. The implementation of this model of feeding by the examined women was induced by the child's independence, due to the accelerated psychomotor development (n = 202; 37.0%), the wish to familiarize the child with various tastes and to shape the sensory diversity (n = 55; 10.1%), the desire to broaden family ties by using the method of eating from the family table (n = 50; 9.1%). The observations made by mothers during the process of dietary expansion with the BLW method were mainly the occurrence of mess (n = 147, 36.0%), the necessity to feed the child (n = 15, 3.7%), food wastage (n = 13, 3.2%), lack of maturity of the child for the BLW method (n = 7, 1.7%) and treating food as a game (n = 3, 0.7%). As many as 45 (11.1%) mothers indicated the occurrence of a food choking incident.

Independent eating by children was indicated by 563 (80.5%) mothers. The child's independent decision concerning what to eat, which is in accordance with the BLW method, is accepted by 434 (62.1%) mothers. 39 (5.6%) mothers do not allow their children to choose what to eat and 15 (2.15%) of them do not allow their children to decide how much to eat (**Table 6**).

As declared by mothers, the basic products which were introduced to the diet with the BLW method included: carrots (n = 646; 92.4%), potatoes (n = 531; 75.9%), apples (n = 503; 71.9%) and bananas (n = 420; 60.0%). Less frequently, cereals, turkey, eggs, yogurt, bread, chicken, fish, and tomato were introduced first (**Table 7**).

The predominant forms of thermal processing that the products introduced into the child's diet were given were: cooking (n = 699; 100%), steaming (n = 455; 65.1%), baking (n = 465; 66.5%), stewing (n = 421; 60.2%).

TABLE 6 | Use of the BLW method and children's independence in making feeding decisions.

	n	%
Using the BLW method in feeding their children (*n* = 545; 100%)		
Yes	408	74.8
Not	137	25.1
Observed behavior during the application of the BLW method (*n* = 408; 100%)		
No disadvantages of using the BLW method	178	43.6
The mess	147	36.0
Choking	45	11.0
The need for feeding	15	3.7
Waste of food	13	3.2
Lack of maturity on the part of the child to use the BLW method	7	1.7
Treating food as play	3	0.7
Self-catering by the child		
Yes	563	80.5
Sometimes	116	16.6
Not	20	2.9
The child's own decision on what to eat		
Yes	434	62.1
Sometimes	226	32.3
Not	39	5.6
Child's own decision on how much to eat		
Yes	597	85.41
Sometimes	87	12.45
Not	15	2.15

TABLE 7 | Products implemented by the BLW method (multiple choice question).

		n	%
Most commonly implemented nutritional products	Carrot	646	92.4
	Potatoes	531	75.9
	Apple	503	71.9
	Banana	420	60.0
Less frequently implemented nutritional products	Groats	197	28.1
	Turkey	137	19.6
	Eggs	136	19.4
	Yogurts	114	16.3
	Bread	105	15.0
	Chicken	102	14.5
	Fish	98	14.0
	Tomato	59	8.4
	Cucumber	46	6.5

Socio-Economic Factors and the Use of the BLW Method

The relationships between the analyzed socioeconomic factors and the use of BLW as a method of child feeding are presented in **Table 8**. Neither education ($p = 0.22$), age of the examined child ($p = 0.43$), nor mother's body weight ($p = 0.57$) affected the decision about the use of BLW. On the other hand, a significant correlation was found between the duration of breastfeeding and the use of BLW ($p = 0.003$); the longer the mother breastfed, the more often she declared the use of BLW when expanding the child's diet.

Declarations on the Use of the BLW Method vs. Its Practical Application

At the same time, it was examined to what extent mothers' declarations concerning the application of the BLW method were reflected in the actual application of the principles of this method: such as giving the child products/meals from the family table to eat ($p = 0.00$), feeding the child with a spoon ($p = 0.00$), allowing the child to eat a meal on its own ($p = 0.00$), allowing the child to decide on its own how much to eat ($p = 0.00$), and what to eat ($p = 0.00$) (**Table 9**).

Despite the declaration of using the BLW method in the process of expanding the child's diet, the majority of mothers and their children used the mixed method. In the examined group of mothers, some of them declared that they did not use the BLW method, but they still implemented some of its elements. A statistically significant relationship was found—those mothers who declared the use of BLW more often use these elements in the process of expanding the child's diet than those who did not make such a declaration.

DISCUSSION

Balanced nutrition, dietary supplementation, and the nutritional status of the pregnant woman are of great importance in terms of the nutritional status of the child at different stages of psychomotor and neurodevelopmental development. Learning the principles of healthy eating starts at an early age. The development of individual food preferences that consist of learning about and getting used to new tastes contributes to the acquisition of healthy eating habits that will bear fruit in the future. An indispensable component for this is the nutritional knowledge of the parents, who introduce meals to their child in the postnatal period (26, 27).

In the author's study, the degree of women's knowledge was characterized by a high level. The analysis of our results allows us to state that the elements from the scope of knowledge concerning BLW: knowledge of the method, terminology, and the validity of its use constituted more than a good level, at the same time taking into account the extension of this scope of issues using the Internet. Comparing the study of Brown and Lee it shows an analogy with the author's analysis because also the degree of the range of skills? was also at a high level, but the subjects were informed by health professionals (28).

The process of introducing complementary products to breastfeeding as well as for babies fed with modified milk is important for the baby's microbiome. This is due to the type of food products that contain natural prebiotics such as fructo-oligosaccharides and galacto-oligosaccharides. By expanding the baby's diet and introducing products such as yogurt, kefir, buttermilk and products fortified with probiotics we influence

TABLE 8 | Relationship between BLW use and sociodemographic factors.

Feature	Variable	Uses BLW *n* (%)	Does not use BLW *n* (%)	Materiality level
Education	Higher	380 (93.1)	122 (89.1)	*P* = 0,22
	Medium	23 (5.6)	14 (10.2)	
	Vocation	4 (1.0)	0 (0.0)	
	Basic	1 (0.2)	1 (0.7)	
Length of breastfeeding	Less than 1 month	21 (5.1)	9 (6.6)	*P* = 0,003
	1–2 months	12 (2.9)	15 (10.9)	
	3–4 months	21 (5.1)	10 (7.3)	
	5–6 months	23 (5.6)	14 (10.2)	
	6–12 months	154 (37.7)	50 (36.5)	
	12–24 months	129 (31.6)	26 (19.0)	
	Over 24 months	48 (11.8)	13 (9.5)	
Age of child tested	0–0.5 years	22 (5.4)	6 (4.4)	*P* = 0,43
	0.5–1 year	18 (4.4)	11 (8.0)	
	1–1.5 years	110 (27.0)	31 (22.6)	
	1.5–2 years	25 (6.1)	7 (5.1)	
	2–2.5 years	169 (41.4)	61 (44.5)	
	2.5–3 years	64 (15.7)	21 (15.3)	
Mother's BMI	Underweight	21 (5.1)	5 (3.6)	*P* = 0,57
	Normweight	279 (68.4)	87 (63.5)	
	Overweight	77 (18.9)	29 (21.2)	
	Obesity	23 (5.6)	13 (9.5)	
	Data not available	8 (2.0)	3 (2.2)	
	Total	408 (100)	137 (100)	

the microbiome. By taking advantage of the regionality of product groups and relying on products such as sauerkraut, pickled cucumbers and other pickles, we promote the formation of an immunomodulatory barrier. Popular Polish regional products such as sauerkraut, pickled cucumbers and fermented milk products contain probiotic bacteria. Additionally, products specially enriched with probiotic bacteria can be purchased on the Polish market. It has been shown that probiotic bacteria, through colonization in the intestines of the host, among other things, reduce the risk of growth of potential pathogenic bacteria and affect the anatomical-physical and microbiological barriers of the gastrointestinal tract, as well as affect the immunity of the macroorganism, including the local immunity of the gastrointestinal tract (29–32). It has been found that different strains of bacteria, even though they belong to the same species, can have different effects on the organism, so when selecting bacteria used in probiotics, it is important to choose those that show good clinical effects (31, 33).

Probiotic bacteria show a wide spectrum of action, including stimulating the mucosa associated lymphoid tissue (MALT) immune system—also called the common mucosal immune system (CMIS) (34, 35). The MALT or CMIS system is formed, among others, by elements of immunity of the gastrointestinal tract (GALT—gut associated lymphoid tissue), respiratory system (BALT—bronchus associated lymphoid tissue), genitourinary associated lymphoid tissue (GUALT), and skin SALT—skin associated lymphoid tissue. The immune response of the MALT system, is characterized by activation of natural and acquired immunity mechanisms, through production of a whole range of cytokines, chemokines and growth factors, as well as immunoglobulins class: G, M, A, including secretory immunoglobulins S-IgA and S-IgM (34–37). As the last mentioned antibodies, min. by coating and agglutinating microorganisms, they prevent their adhesion to the gastrointestinal epithelium, although they also show regulatory effects, including through S-IgM antibodies, on elements of the immune system. Thus, the MALT system shows not only a combative or destructive effect against bacteria, viruses, fungi and parasites, but also causes neutralization of bacterial and fungal toxins Probiotics show a significant effect on the elements of the immune system in the gastrointestinal tract (GALT), which results in increased local, but also general immunity, which in turn leads to a reduction in the impact of infections, including bacterial, viral and other on the macroorganism. This effect is combined with and depends on the appropriate strain of bacteria used in the probiotic and this is, it seems, crucial for a good effect in the use of probiotics, although its effect is also influenced by its dose and time of administration, but also by the immune status of the macroorganism (30, 31, 36–41).

WHO and ESPGHAN recommend exclusive breastfeeding at least until the child is 6 months old, whereas after 6 months of age and between 17 and 26 weeks of age, according to PTGHiZD,

TABLE 9 | Assumptions of the BLW method used and not used in the group of mothers declaring and not declaring to use the BLW method.

Feature	Variable	Uses BLW *n* (%)	Does not use BLW *n* (%)	Materiality level
Giving the child food from the family table to eat	Yes	312 (76.5)	83 (60.6)	*P* = 0,00
	Sometimes	89 (21.8)	39 (28.5)	
	Not	7 (2.9)	15 (10.9)	
Feeding the baby with a spoon	Yes	251 (61.5)	31 (22.6)	*P* = 0,00
	Sometimes	145 (35.5)	6 (4.4)	
	Not	12 (2.9)	0 (0.0)	
Allowing the child to eat alone	Yes	358 (87.7)	87 (63.5)	*P* = 0,00
	Sometimes	52 (12.7)	43 (31.4)	
	Not	2 (0.5)	7 (5.1)	
Allowing the child to decide for him/herself what to eat	Yes	310 (76.0)	48 (35.0)	*P* = 0,00
	Sometimes	95 (23.3)	67 (48.9)	
	Not	3 (0.7)	22 (16.1)	
Allowing the child to decide how much to eat	Yes	366 (89.7)	112 (81.8)	*P* = 0,00
	Sometimes	39 (9.6)	19 (13.9)	
	Not	3 (0.7)	6 (4.4)	
	Total	408 (100)	137 (100)	

the use of supplementary products is allowed according to individual decision and needs of the mother and child (26, 42). The knowledge of the principles of dietary expansion of infants translates into the level of frequency of its application. Our study shows the reflection of knowledge in the frequency of BLW implementation by mothers. The universality of this form of dietary expansion is confirmed by the results of a study by other authors, which also allowed to demonstrate a relationship between a higher level of education and an increased frequency of using the method of introducing solid products (28).

Nutritional patterns reflected in the parents' diet are reproduced and implemented in children's nutrition. The mechanism of metabolic programming has been shown to influence the pathomechanism of the development of metabolic and diet-related diseases, the regulation of leptin and ghrelin synthesis in the hypothalamus, and the nutritional status of the child (43, 44). Based on scientific reports, the timing of administration of specific products is important to the occurrence of later eating habits. In the case of sweet products, they should be introduced as late as possible and at least meet 5% of the energy value of the whole-day ration, due to strong taste habits. The researchers Townsend and Pitchford proved the positive influence on health-promoting habits in the case of using the BLW method in comparison with children not eating this way, additionally demonstrating in them an increased inclination to sweet products (14, 45). Additionally, the authors Brown and Lee emphasize the positive and safe quality of food prepared using the BLW method (28).

Our results show the predominance of the mixed form of complementary feeding and the lower frequency of the full BLW method. Among mothers who did not use this method, an increased tendency not to give products from the family table was observed, as well as a more frequent lack of parents' consent to decide independently about the quantity and quality of eaten products.

The analysis of the author's study shows that the dietary model was expanded to include vegetables, fruits in the first place, using favorable thermal treatment including methods such as boiling, steaming, baking, stewing which are the correct techniques for preparing meals according to the nutritional recommendations for children (26).

The variety of controversies arising around the BLW method is conditioned by the disadvantages and advantages attributed to this way of nutrition. The disadvantages include chaos during meal consumption, the possibility of choking, increase in saturated fat supply, nutritional deficiencies, insufficient coverage of nutritional fiber requirements, wasting of food (26, 28). The increased incidence of choking, which is the main concern among parents and health care professionals, is not confirmed according to scientific reports, as it occurs similarly in spoon-fed children and is regulated by the defense mechanism of the developed unconditioned reflex To avoid choking, check the food before serving to make sure it is soft enough. Avoid food that forms crumbs in the mouth or that can stick to the palate. The child should eat at his own pace and under his own control—no one should force the child to eat more quickly. Small foodstuffs, such as chickpeas, grapes and blueberries, should always be mashed first and cut up properly. It is also forbidden to give baby nuts whole, but blended nuts in the form of nut butter, cashew butter or walnut butter can be given. The child should have a stable sitting position while eating—it should not lean back or to the sides. And an adult should always be with the child (45–47).

Among the elements inducing the implementation of BLW, according to the studies of other researchers and in the author's study, the following are important: expansion of family ties through the use of the family table method, the pleasure of meal consumption, the formation of healthy eating habits (increased supply of vegetables, fruit, whole grain products), the taste and organoleptic diversity, the formation of independence in food consumption and the formation of self-control in the relationship with food and, above all, the feeling of satiety (28, 48, 49). It is also worth noting that in our study it was observed that there is a percentage of mothers who, despite the lack of declaration of using the BLW method with their child, implement in feeding their children elements which are a departure from the traditional model of nutrition. Such a relationship is also demonstrated in the study by Brown (50).

It is necessary to implement continuous nutritional education on the nutrition of infants, children, as well as women planning pregnancy, with ongoing pregnancy and during lactation, including elements of psychodietetics based, for example, on motivational dialogue. The principles of anti-inflammatory dietoprophylaxis used among people trying to have a baby are common. The typical anti-inflammatory diet emphasizes fruits, vegetables, lean protein, nuts, seeds, and omega-3 fatty acids. Many products are eliminated from the diet, incl. Foods high in omega-6 fatty acids include: high-fat dairy products (such as milk, cheese, butter, and ice cream), margarine, meats, peanuts. It is important that parents follow healthy eating principles when expanding their child's diet rather than restrictively eliminating foods. Such restrictions may predispose to food aversion, food neophobia and even to avoidant/restrictive food intake disorder (AFRID) (51, 52).

CONCLUSIONS

The level of women's knowledge in the scope of dietary expansion of infants by the BLW method is high, taking into account the necessity of continuous nutritional education. The use of the mixed method of dietary expansion is dominant among the mothers participating in the study. The frequency of implementation of the BLW method depended on the level of education; women with higher education use it more often. Among the positive aspects of using the BLW method, the women surveyed indicate the child's independence, while among the disadvantages, mess, and chaos. There is a group of mothers who, despite not being identified with the BLW method, use its elements in the child's everyday feeding, such as the child deciding on its own how much and what to eat.

LIMITATIONS OF THE STUDY

The results of the study should be interpreted taking into account its limitations. Limitations include the low heterogeneity of the study group in terms of age (predominance of women aged 30–35) and education level (predominantly higher education) despite the random selection of nurseries and clinics where data collection took place. Our study was a retrospective study, which may influence the occurrence of false memory effect, especially in the group of mothers of children aged 2–3 years.

The advantage of our study is the size of the study group–699 qualified mothers with their children and the random selection of nurseries and pediatric outpatient clinics where this study was conducted. It is also worth mentioning here that very few studies on this topic have been conducted so far.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee of the Medical University of Silesia in Katowice (No. KNW/0022/KB1/151/I/11). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AB-D and PT: conceptualization. PT, AB-D, and MGro: investigation. AB-D, MGro, and MGra: writing-original draft preparation: AB-D, MGro, MGra, ES, and OK: writing-review and editing. AB-D: visualization. ES and OK: supervision. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Pietrobelli A, Agosti M. Nutrition in the first 1000 days: ten practices to minimize obesity emerging from published science. *Int J Environ Res Public Health*. (2017) 12:1491. doi: 10.3390/ijerph14121491
2. Fitria Utami A, Wanda D. Is the baby-led weaning approach an effective choice for introducing first foods? A literature review. *Enferm Clin*. (2019) 2:87–95. doi: 10.1016/j.enfcli.2019.04.014
3. D'Auria E, Bergamini M, Staiano A. Baby-led weaning: what a systematic review of the literature adds on. *Ital J Pediatr*. (2017) 44:49. doi: 10.1186/s13052-018-0487-8
4. Szajewska H, Socha P, Horvath A. Zasady zywienia zdrowych niemowlat. Stanowisko polskiego towarzystwa gastroenterologii, hepatologii i zywienia dzieci [Principles of nutrition of healthy infants. Position statement of the polish society of gastroenterology, hepatology, and child nutrition]. *Pediatria*. (2021) 11:321–338. doi: 10.17444/SMP2021.18.02
5. Fewtrell M, Bronsky J, Campoy C. Complementary feeding: a position paper by the european society for paediatric gastroenterology, hepatology, and nutrition (ESPGHAN) committee on nutrition. *J Pediatr Gastroenterol Nutr*. (2017) 64:119–32. doi: 10.1097/MPG.0000000 0001454
6. WHO (World Health Organization). *Complementary Feeding. Report of the Global Consultation and Summary of Guiding Principles*. Geneva: WHO (World Health Organization) (2002). Available online at: http://www.who.int/nutrition/publications/Complementary_Feeding.pdf (accessed December 10–13, 2001).
7. Schwarzenberg SJ, Georgieff MK, Committee on Nutrition. Advocacy for improving nutrition in the first 1000 days to

support childhood development and adult health. *Pediatrics*. (2018) 141:e20173716. doi: 10.1542/peds.2017-3716

8. Cattaneo A, Fallon M, Kewitz G, Mikel-Kostyra K, Robertson A. *Zywienie niemowlat i małych dzieci: Standardy Postepowania dla Unii Europejskie*j (2007). Available online at: http://cnol.kobiety.med.pl/wp-content/uploads/2019/01/standardy_zywienia_EU.pdf (accessed March 1, 2022).
9. Neves FS, Romano BM, Campos A, Pavam CA, Oliveira R, Cândido A, et al. Brazilian health professionals' perception about the Baby-Led Weaning (BLW) method for complementary feeding: an exploratory study. *Rev Paul Pediatr.* (2021) 40:e2020321. doi: 10.1590/1984-0462/2022/40/2020321
10. D'Andrea E, Jenkins K, Mathews M, Roebothan B. Baby-led weaning: a preliminary investigation. *Can J Diet Pract Res.* (2016) 77:72–7. doi: 10.3148/cjdpr-2015-045
11. Cameron SL, Heath AL, Taylor RW. Healthcare professionals' and mothers' knowledge of, attitudes to and experiences with, baby-led weaning: a content analysis study. *BMJ Open.* (2012) 2:e001542. doi: 10.1136/bmjopen-2012-001542
12. Krupa-Kotara K. *Dietetyka pediatryczna, Rozdział 2.5. Rozszerzanie diety niemowlecia w aspekcie praktycznym.* Wyd. Slaski Uniwersytet Medyczny (2021). p. 98–107
13. Cameron LS, Taylor WR. Heath MAL. Development and pilot testing of baby-led introducing to solids- a version of baby-led weaning modified to address concerns about iron deficiency, growth faltering and choking. *BMC Pediatr.* (2015) 15:99. doi: 10.1186/s12887-015-0422-8
14. Williams Erickson L, Taylor WR, Haszard JJ. Impact of a modified version of baby-led weaning on infant food and nutrient intakes: the BLISS randomized controlled trial. *Nutrients.* (2018) 10:740. doi: 10.3390/nu10060740
15. Jastrzebska I. *What Do Happy Beavers Eat?* Wyd. Zywieniowo (2020).
16. Daniels L, Heath AL, Williams SM, Cameron SL, Fleming EA, Taylor BJ, et al. Baby-led introduction to solids (BLISS) study: a randomised controlled trial of a baby-led approach to complementary feeding. *BMC Pediatr.* (2015) 15:179. doi: 10.1186/s12887-015-0491-8
17. Ventura AK. Does breastfeeding shaoe food preferences links to obesity. *Ann Nur Metab.* (2017) 3:8–15. doi: 10.1159/000478757
18. Ventura KA, Worobey J. Early influences on the development of food preferences. *Curr Biol.* (2013) 23:R401–8. doi: 10.1016/j.cub.2013.02.037
19. Morison JB, Heath MAL, Haszard JJ. Impact of a modified version of baby-led weaning on dietary variety and food preferences in infants. *Nutrients.* (2018) 10:1092. doi: 10.3390/nu10081092
20. Morison JB, Taylor WR, Haszard JJ. How different are baby-led weaning and conventional complementary feeding? A cross-sectional study of infants aged 6–8 months. *BMJ Open.* (2016) 6:e010665. doi: 10.1136/bmjopen-2015-010665
21. Wyn Jones S, Lee M, Brown A. Spoonfeeding is associated with increased infant weight but only amongst formula-fed infants. *Matern Child Nutr.* (2020) 16:e12941. doi: 10.1111/mcn.12941
22. Brown A, Lee M. An exploration of experiences of mothers following a baby-led weaning style: developmental readiness for complementary foods. *Matern Child Nutr.* (2013) 9:233–43. doi: 10.1111/j.1740-8709.2011.00360.x
23. Fangupo LJ, Heath AL, Williams SM, Erickson Williams LW, Morison BJ, Fleming EA, et al. A baby-led approach to eating solids and risk of choking. *Pediatrics.* (2016) 138:e20160772. doi: 10.1542/peds.2016-0772
24. Obligation of the Session Marshal of the Republic of Poland. Of 18 June 2020 on the publication of the uniform text of the Act—Labour Code Warszawa (2020). Available online at: https://www.pip.gov.pl/pl/f/v/224803/D2020000132001.pdf (accessed July 30, 2020).
25. Social Insurance Institution. *Fathers on Maternity Benefit.* Available online at: https://www.zus.pl/o-zus/aktualnosci/-/publisher/aktualnosc/0/ojcowie-na-zasilku-macierzynskim/4070408 (accesed March 1, 2022).
26. Białek-Dratwa A, Soczewka M, Grochowska-Niedworok E. Expanding an infant's diet using the baby-led weaning method ("bobas likes choice"). *Pediatr Fam Med.* (2020) 16:362–7. doi: 10.15557/PiMR.2020.0065
27. D'souza N, Behere RV, Patni B, Deshpande M, Bhat D, Bhalerao A, et al. Pre-conceptional maternal vitamin b12 supplementation improves offspring neurodevelopment at 2 years of age: priya trial. *Front Pediatr.* (2021) 9:755977. doi: 10.3389/fped.2021.755977
28. Brown A, Lee M. A descriptive study investigating the use and nature of baby-led weaning in a UK sample of mothers. *Matern Child Nutr.* (2011) 34–47. doi: 10.1111/j.1740-8709.2010.00243.x
29. Gajewska J, Błaszczyk MK. Probiotyczne bakterie fermentacji mlekowej. *Post Mikrobiol.* (2012) 51:55–65.
30. Górska S, Jarzab A, Gamian A. Bakterie probiotyczne w przewodzie pokarmowym człowieka, jako czynnik stymulujacy układ odpornościowy. *Post Hig Med Dośw.* (2009) 63:653–67.
31. Kubiszewska I, Januszewska M, Rybka J, Gackowska L. Bakterie kwasu mlekowego i zdrowie: czy probiotyki sa bezpieczne dla czlwieka? *Post Hig Med Dośw.* (2014) 68:1325–34.
32. Sliwa-Dominiak J, Deptuła W. Mikroorganizmy komensaliczne u ssaków—wybrane dane. *Medycyna Wet.* (2010) 66:383–8.
33. Jach M, Łoś R, Maj M, Malm A. Probiotyki—aspekty funkcjonalne i technologiczne. *Post Mikrobiol.* (2013) 52:161–70.
34. Ray A, Dittel BN. Interrelatedness between dysbiosis in the gut microbiota due to immunodeficiency and disease penetrance of colitis. *Immunology.* (2015) 146:359–68. doi: 10.1111/imm.12511
35. Sivan A, Corrales L, Hubert N, Williams JB. Aquino-Michaels K, Earley ZM, et al. Commensal bifidobacterium promotes antitumor immunity and faciliyayes anti-PD-L1 efficacy. *Science.* (2015) 350:1084–9. doi: 10.1126/science.acc4255
36. Garg AD, Agostinis P. Molecular and translational classifications of DAMPs in immunogenic cell death. *Front Immunol.* (2015) 8:588. doi: 10.3389/fimmu.2015.00588
37. Vieira AT, Teixeira MM, Martins FS. The role of probiotics and prebiotics in inducing gut immunity. *Front Immunol.* (2013) 4:445. doi: 10.3389/fimmu.2013.00445
38. Iwasaki A. Exploiting mucosal immunity for antiviral vaccines. *Annu Rev Immunol.* (2016) 34:575–608. doi: 10.1146/annurev-immunol-032414-112315
39. Lavin Y, Mortha A, Rahman A, Merad M. Regulation of macrophage development and function in peripheral tissues. *Nat Rev Immunol.* (2015) 15:731–44. doi: 10.1038/nri3920
40. Vong L, Lorentz RJ, Assa A, Glogauer M, Sherman PM. Probiotic lactobacillus rhamnosus inhibits the formation of neutrophil extracellular traps. *J Immunol.* (2014) 192:1870–7. doi: 10.4049/jimmunol.1302286
41. You J, Dong H, Mann ER, Knight SC, Yaqoob P. Probiotic modulation of dendritic cell function is influenced by ageing. *Immunobiology.* (2014) 219:138–48. doi: 10.1016/j.imbio.2013.08.012
42. Brown A, Lee M. Early influences on child satiety—responsiveness: the role of weaning style. *Pediatr Obes.* (2015) 10:57–66. doi: 10.1111/j.2047-6310.2013.00207.x
43. Kim-Herrera EY, Ramírez-Silva I, Rodríguez-Oliveros G, Ortiz-Panozo E, Sánchez-Estrada M, Rivera-Pasquel M, et al. Parental feeding styles and their association with complementary feeding practices and growth in mexican children. *Front Pediatr.* (2021) 9:786397. doi: 10.3389/fped.2021.786397
44. Sámano R, Chico-Barba G, Martínez-Rojano H, Hernández-Trejo M, Birch M, López-Vázquez M, et al. Factors associated with weight, length, and bmi change in adolescents' offspring in their first year of life. *Front Pediatr.* (2021) 9:709933. doi: 10.3389/fped.2021.709933
45. Townsend E, Pitchford N. Baby knows best? The impact of weaning style on food preferences and body mass index in early childhood in a case-controlled sample. *BMJ Open.* (2012) 2:e000298. doi: 10.1136/bmjopen-2011-000298
46. Cameron S, Heath A, Taylor R. How feasible is baby-led weaning as an approach to infant feeding? A review of the evidence. *Nutrients.* (2012) 4:1575–609. doi: 10.3390/nu4111575
47. Fu X, Conlon CA, Haszard JJ, Beck KL, von Hurst PR, Taylor RW, et al. Food fussiness and early feeding characteristics of infants following Baby-Led Weaning and traditional spoon-feeding in New Zealand: An internet survey. *Appetite.* (2018) 130:110–6. doi: 10.1016/j.appet.2018.07.033
48. Lipsky LM, Haynie DL, Liu D, Chaurasia A, Gee B, Li K, et al. Trajectories of eating behaviors in a nationally representative cohort of U.S. adolescents during the transition to young adulthood. *Int J Behav Nutr Phys Act.* (2015) 12:138. doi: 10.1186/s12966-015-0298-x
49. Camacho-Morales A, Caba M, García-Juárez M, Caba-Flores MD, Viveros-Contreras R, Martínez-Valenzuela C. Breastfeeding contributes to physiological immune programming in the newborn. *Front Pediatr.* (2021) 9:744104. doi: 10.3389/fped.2021.744104

50. Brown A. Differences in eating behavior, well-being and personality between mothers following baby-led vs. traditional weaning styles. *Matern Hild Nutr.* (2016) 12:826–37. doi: 10.1111/mcn.12172
51. Sikora DM. The treatment of avoidant/restrictive food intake disorder (ARFID) with a predominance of anxiety presentation. A proposal of a protocol for the therapeutic procedure. *Psychotherapy*. (2021) 198:33–4. doi: 10.12740/PT/141443
52. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab*. (2012) 2012:539426. doi: 10.1155/2012/539426

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Black Soldier Fly Larvae Meal in the Diet of Gilthead Sea Bream: Effect on Chemical and Microbiological Quality of Filets

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The chemical and microbiological characteristics of filets of *Spaurus aurata* L. specimens fed with diets containing a *Hermetia illucens* meal (HIM) at the 25, 35, and 50%, as a partial replacement for fish meal (FM) were evaluated. The diets, formulated to satisfy the nutritional needs of fish, were isoenergetic (22 MJ/kg gross energy), isonitrogenous (43 g/100 g, a.f.), and isolipidic (19 g/100 g, a.f.). Seventy-two specimens were randomly killed after 186 days of growing trials. Then, the filets were analyzed for chemical profile, fatty acids, amino acids, minerals, and microbial flora. Data were subjected to statistical analysis. No significant differences were observed in chemical composition. The sum of polyunsaturated fatty acids (PUFAs) showed a similar content in the filets; eicosapentaenoic acid was similar in the filets of HIM0, HIM35%, and HIM50%, whereas docosahexaenoic acid was higher in filets of the HIM0 group. n3/n6 PUFA ratio and the sum of EPA + DHA showed a high value ($p < 0.001$) in filets of the group fed with FM. No significant difference was observed in thrombogenic index and hypocholesterolaemic/hypercholesterolaemic ratio in the groups; the atherogenic index showed a higher value ($p = 0.001$) in the HIM50% group. Indispensable amino acids showed some significant ($p < 0.0001$) differences in the groups; arginine and phenylalanine content was higher in the filets of fish fed with FM; isoleucine and valine content was higher in the filets of HIM50%; leucine, lysine and methionine content was lower in the filets of HIM35%; histidine content was lower in the filets of HIM25%; tryptophan content was lower in filets of the HIM50% group. EAA/NEAA ratio showed highest value in the filets of the group that received FM. The presence of HIM in the three diets kept chromium, manganese, iron, copper, zinc, and nickel levels lower than those recommended by various authorities. Ca/P ratio showed a higher level ($p < 0.0001$) in the group fed with FM than those fed with diets containing HIM. The insect meal in the diets did not influence the microbiological profile of fish. Use of HIM as an unconventional feed ingredient in *Sparus aurata* diet looks promising, although the quality of filets may be affected.

Keywords: nutritional quality, mineral profile, microbiological quality, seafood, insect meal

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INTRODUCTION

The global demand and consumption of fish are increasing to meet the needs of the growing population at a faster rate than the demand for fish feed ingredients; this is leading to a rapid decline in fish meal (FM) availability and simultaneous rise in prices (1, 2). FM is the optimal animal protein source used in commercial fish feeds (3), with high bioavailability of nutrients and an adequate nutritional composition, which meets the requirements of essential amino acids and fatty acids of fish species (4, 5).

However, the use of FM is unsustainable from both environmental and economic points of view.

The aquaculture industry's most positive contribution is the search for alternative ingredients that are integrated into sustainable farming systems and provide high quality protein and lipids without negatively impacting fish health, performance, and disease resistance (6, 7), and without compromising the nutritional value of farmed fish for humans (8, 9). The use of nonconventional ingredients such as insect materials with nutritional and nutraceutical potential for human and animal health has been proposed as a relevant sustainable element of the agri-food chain (10–12). Insect meal (IM) is considered an adequate protein and lipid source that can be used as a substitute for FM in aquaculture feed because of its protein, amino acid, and fatty acid profiles (13). However, the use of insects as feed is a relatively new practice on a commercial scale, and many questions remain to be tackled: (i) the risk of transfer of pathogens into the production system (14) so much so that EFSA identifies the substrate used to feed insects as the key entry point for contamination (15); (ii) the optimal level of food replacement of FM for IM, which can vary considerably from 25 to 100% because of different compositions of larvae, fish species, and diets (16).

Among different insect species considered for possible use in aquaculture, *Hermetia illucens* is one of the most interesting because its sustainability is linked to its abilityto convert food waste materials or manure into high-quality insect nutrients (17). The proximate composition of the *H. illucens* meal (HIM) is highly variable; based on dry matter, protein and lipid contents were reported for de-fatted HIM of 47.2 and 11.8% (18) and 51.8 and 14.8% (19), respectively, and for full-fat HIM of 36.2 and 18%, respectively (20). Ash content ranged between 11 and 15% with high mineral concentrations characterized by high Ca/P ratio (21).

Although studies on the use of black soldier fly larvae meal in fish finishing started in 1987 (22), it became popular again, especially after 2017 when the European Commission allowed the use of proteins derived from 7 species of insects as alternative protein sources for aquafeed formulation (23). Previous studies have shown that replacing FM with IM in fish feeds results in changes in filet quality (24), without adversely affecting fish growth (25, 26). In particular, one problem for both producers and consumers is the consequent decrease in alfa-linolenic acid (ALA; C18:3n3), eicosapentaenoic acid (EPA; C20:5n3), and docosahexaenoic acid (DHA; C22:6n3) levels in fish filets due to the inclusion of HIM in the feed (27, 28). However, it may be possible to modify the nutritional composition of *H. illucens* through the feeding media of the insect (29–31), as observed for *H. illucens*-fed fish offal and algae where significant amounts of EPA and DHA were found (27, 32).

This study is part of a much larger research project, "Feed Insects For Aquaculture" (FIFA), which aims to reveal the nutritional value of a protein-rich insect meal (IM) produced from *H. illucens* larvae and used as a partial substitute of fish meal in *Sparus aurata* feeding. In a previous study, the proximate, fatty acid, amino acid, and mineral compositions, microbiological profile, and organoleptic characteristics of four diets formulated for *Sparus aurata* and containing 25, 35, and 50% of defatted HIM (33) as replacement for FM were characterized. Given the growing interest in HIM as an alternative protein source to replace FM in fish feeds, in another study on *Sparus aurata* fed with the diets described above, the growth performance and feed utilization efficiency were reported (not yet published), and the organoleptic properties of the filets were analyzed using a sensor-based instrument platform consisting of E-eye, E-nose with 18 MOS sensors, and a potentiometric E-tongue with 7 chemical sensors (34). This study mainly focussed on the chemical and microbiological characteristics of filets of *Spaurus aurata* L. fed with diets containing increasing levels of HIM as a partial replacement for FM.

MATERIALS AND METHODS

The experimental protocol was designed according to the Italian legislation (35) and guidelines of the current European Directive (36) on the protection of animals utilized for scientific purposes. The experimental protocol was authorized by the Italian Ministry of Health (Ministerial Authorization number 491/2019-PR released on 4 July 2019).

Diet Formulation

All the diets were developed to meet the nutritional requirements of *Sparus aurata* and beisoenergetic (about 22 MJ/kg gross energy), isonitrogenous (about 43 g/100, as fed), and isolipidic (about 19 g/100, as fed). A control formula (HIM0) containing fish meal (FM) as an exclusive protein source of animal origin was developed. For the other three diets (HIM25, HIM35, HIM50%), the defatted *Hermetia illucens* meal was added at 25, 35, and 50% (as fed basis) to the control formula, replacing FM, to create three formulations characterized by different amounts of HIM (79, 110, and 157 g/kg). The other ingredients of the diets were adjusted to obtain iso-energetic formulas.

The diets were prepared by SPAROS Lda (Olhao, Portugal), which was commissioned to prepare the extruded fish diets. The ingredients were weighed, mixed, and grounded, and the feeds were extruded in a single screw extruder using a die diameter (d_d) of 4 mm; after extrusion, kibbles were dried and coated with oil. The ingredients and proximate composition of the diets (HIM0, HIM25, HIM35, and HIM50%) are reported in **Table 1**. The fatty acid, amino acid, and mineral compositions, and microbiological profile were previously reported by Oteri et al. (33).

TABLE 1 | Ingredients and proximate composition of the experimental diets.

	DIET			
	HIM0	**HIM25%**	**HIM35%**	**HIM50%**
Ingredients, % as fed				
Fish meal	25.00	18.75	16.25	12.50
Hermetiaillucens meal	0	7.90	11.00	15.70
Soy protein concentrate	5.00	5.00	5.00	5.00
Wheat gluten	5.00	5.00	5.00	5.00
Corn gluten	5.00	5.00	5.00	5.00
Soybean meal 48	15.00	15.00	15.00	15.00
Rapeseed meal	5.00	5.00	5.00	5.00
Wheat meal	17.45	15.17	14.21	12.88
Whole peas	4.00	4.00	4.00	4.00
Fish oil	5.00	5.00	5.00	5.00
Rapeseed oil	10.00	9.80	9.80	9.80
Vitamin and mineral premix	1.00	1.00	1.00	1.00
Vitamin C35	0.03	0.03	0.03	0.03
Vitamin E50	0.02	0.02	0.02	0.02
Antioxidant	0.30	0.30	0.30	0.30
Sodium propionate	0.10	0.10	0.10	0.10
MCP, monocalcium phosphate	1.50	2.20	2.50	2.80
L-Lysine	0.30	0.35	0.37	0.40
L-Tryptophan	-	0.03	0.04	0.05
DL-Methionine	0.10	0.15	0.18	0.22
L-Taurine	0.20	0.20	0.20	0.20
Proximate analysis, g/100g as fed				
Dry matter	92.33	92.78	92.90	92.64
Crude protein	42.7	42.7	42.7	42.7
Crude fat	18.6	18.6	18.6	18.7
Crude fiber	2.3	2.2	2.2	2.1
Ash	9.3	9.3	9.4	9.3
NFE*	19.43	19.98	20.00	19.84

HIM0, fish meal; HIM25, HIM35, and HIM50%, Hermetia illucens meal at the 25, 35, and 50% substitution rates of fish meal, respectively.

**Nitrogen-free extract, NFE (g/100g) = 100–(crude protein + crude fat + crude fiber + ash).*

Feeding Trial and Facilities

The experimental study on *Sparus aurata* specimens was carried out at the IRBIM facility in the CNR headquarter in Messina (Italy). On 3 February 2020, 332 fish purchased by the Ittica Caldoli Company (Lesina, Foggia, Italy) were transported to the IRBIM-CNR facility and transferred to a large tank (4.5 m^3) for about 1 week to acclimatize to the breeding conditions. During that time, fish were fed with a commercial diet (46% protein, 16% fat; 20.7% NFE, 2.3% crude fiber; Aller Blue Omega 3 mm; Aller Aqua Company, Christiansfeld, Denmark). After 1 week of acclimation, a total of 324 mixed-sex specimens were individually weighed (average initial weight: 143.65 ± 25.94 g) and randomly divided into 12 indoor fiberglass tanks of 1.4 m^3 (27 fish/tank, 3 replicate tanks per diet, and total of 81 fish per diet), in an open circuit system, with intake and discharge of 12 L/min of water from and to the sea (12 complete tank renewals a day). Some water parameters (pH, O_2, temperature) were monitored daily using a professional multi-parametric probe (YSI Professional Plus Multi-Parameters Water Quality Meter probe; Xylem Inc., Yellow Springs, OH, United States). The fish were fed with the commercial diet and adapted for a further 7 days to the experimental conditions.

Twice a day (at 9:00 and 16:00 h) and 6 days a week, or for over 180 days (18 February−24th August), the fish were fed with the experimental feeds (HIM0, HIM25, HIM35, and HIM50%), initially at 0.8% and with an increase of up to 1.5% of body weight depending on water temperature. Throughout the growth trial period, tank biomass was weighed in bulk every 20 days to update daily feed ration. The tanks were inspected daily for mortality, which was, throughout the duration of the experiment,.003%.

At the end of the trial, all the fish were starved for 24 h and killed by overdose (500 mg/L) with an anesthetic (tricaine methanesulfonate solution, MS-222; Sigma-Aldrich, Italy); body weight (390 ± 49, on average) and length (28 ± 49 cm on average) were determined individually for all the fish. A subsample of 72 specimens ($n = 18$ fish per diet and 6 fish/tank) was randomly slaughtered and transported, in dry ice to the Laboratories of the Department of Veterinary Sciences-Unit of Animal Production, University of Messina (Messina, Italy), where they were gutted, fileted, deskinned, sampled in small aliquots, vacuum-packed, and freeze-dried prior to being subjected to scheduled analyses. Then, each aliquot of the filets was defrosted and homogenized with a common laboratory knife mill (Grindomix GM 200; Retsch GmbH, Haan, Germany) for the analyses described in section 2.3.

A total of 36 specimens ($n = 9$ fish per diet and 3 fish/tank) were randomly slaughtered and transported in sterile plastic bags in dry ice to the Laboratories of the Department of Veterinary Sciences–Unit of Inspection of Food of Animal Origin, University of Messina (Messina, Italy) and processed within 3 h.

Analyses of Chemical Composition of Fish Muscle

The proximate composition of the ground filets from the four groups of fish (total number = 72; 18 fish per diet and 6 fish per replication) was determined following the AOAC (37) methods for moisture (method 950.46), crude protein (method 981.10), and ash (method 920.153).

For determination of total lipid n, the aliquots (approximately 2 g) of wet filet muscles from the four fish groups ($n = 72$) were ground, and the oil was extracted using a chloroform/methanol (2:1, v/v) solution (38). Each chemical analysis was performed in triplicate. Then, total lipids were used to prepare fatty acid methyl esters (FAMEs) for the analysis of fatty acid (FA) profile, according to the method of Christie (39). In particular, on each sample of total lipid, 2 ml of methanol:sulfuric acid (9:1, *v/v*) solution was added, and the mixture was heated at 100°C for 1 h. The FAMEs were analyzed with a Trace 1310 chromatograph (Thermo Fisher Scientific, Milan, Italy) provided with a flame ionization detector (FID). Separation of FAMEs was carried out using a 30 m ×.25 mm (length × internal diameter). fused silica capillary column

(Omegawax 250; Supelco, Bellefonte, PA, United States) 25-µm film, and maintained at 100°C for 5 min, from 100 to 240°C at 4 °C/min and a final isotherm of 20 min at 240°C. Injector and detector temperatures were set at 250°C. Injection volume and split ratio were 0.5 µl and 1:50, respectively. The carrier gas, helium (He), was set at a flow rate of 1 ml/min. Data acquisition and processing were performed using Chromeleon™ Software (Thermo Fisher Scientific, Milan, Italy). Fatty acids of the fish samples were identified by comparing the relative retention times of FAMEs with those of a standard mix solution (mix 37 FAMEs; Supelco, Inc., Bellefonte, PA, United States) under the same analytical conditions. FA concentrations were expressed as g/100 g, where 100 g was the total of all areas of the identified FAMEs. Nutritional indices that consider different fatty acids according to their different contributions to the promotion or prevention of cardiovascular disorders were calculated from the identified fatty acids. Atherogenic (AI) and thrombogenic (TI) indices were calculated using the Ulbricht and Southgate equations (40), while hypocholesterolemic/hypercholesterolemic ratio (H/H), was calculated using the equation proposed by Santos-Silva et al. (41). The three indices were calculated as follows:

$$IA = [C12:0 + (4 \times C14:0) + C16:0]/(\sum n6 - PUFA + \sum n3 - PUFA + \sum MUFA) \quad (1)$$

$$IT = (C14:0 + C16:0 + C18:0)/[(.5 \times \sum MUFA) + (.5\times \sum n6 - PUFA) + (3 \times \sum n3 - PUFA) + (\sum n3 - -PUFA/\sum n6 - PUFA)] \quad (2)$$

$$H/H = (C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3)/(C14:0 + C16:0) \quad (3)$$

Moreover, the peroxidation index (PI), that expresses a measure of peroxidation susceptibility and peroxidative lipid damage for a particular phospholipid membrane, was calculated using the following formula reported by Luciano et al. (42):

$$PI = (\%dienoic\times 1) + (\%trienoic \times 2) + (\%tetraenoic \times 3) + (\%pentaenoic \times 4) + (\%hexaenoic \times 5) \quad (4)$$

For amino acid profile, aliquots (approximately.25 g) of wet filet muscles from the four fish groups ($n = 72$) were hydrolyzed in 10 ml of an HCl solution (6M) at 110°C for 24 h. During acid hydrolysis, asparagine and glutamine were converted to aspartic and glutamic acids (43); therefore, they were calculated as the sum of aspartic acid plus asparagine and of the glutamic acid plus glutamine. For cysteine analysis (43), an oxidation reaction using performic acid was performed for deamination prior to acid hydrolysis with an HCl solution (6M). For tryptophan analysis, acid hydrolysis was performed using 10 ml of a NaOH solution (4M) at 112°C for 16 h; then, cooling and neutralization of each sample were performed with acetic acid (44). Amino acids were analyzed with a Trace 1310 chromatograph (Thermo Fisher Scientific, Milan, Italy) provided with a flame ionization detector (FID) and a ZB-AAA Amino Acid column (10 m × 0.25 mm ID); oven temperature was programmed from 110 to 320°C at 32 °C/min, with a final isotherm of 320 °C (1 min). Injector and detector temperatures were 250 and 320°C, respectively. Injection volume and split ratio were 2.5 µl and 1:15, respectively. Procedures for purification, pre-column derivatization, and quali-quantitative analyses of each amino acid were performed using EZ:Faast Kit (Phenomenex, Torrance, CA, United States).

TABLE 2 | Analytical line length (nm) utilized for analysis.

Element	nm	Element	nm
Cr	267.716	Se	196.026
Cu	327.393	Ni	231.064
Fe	238.204	Mn	257.610
K	766.490	Zn	213.857
P	213.617	Mg	285.592
Na	589.592	Ca	317.933

The mineral profile of fish samples, deprived of bones and scales, was analyzed with a high-performance dispersing instrument. About 0.5 g of the sample filets were exactly weighed in a pre-cleaned PTFE vessel by acidic wash and then digested with 7 ml of 69% Nitric acid TraceSelect and 1 ml of H_2O_2 at 30% (Optima™ for Ultra Trace Analysis), both purchased from Honeywell Fluka (Seelze, Germany). The closed vessels were introduced into a microwave digestion system (Ethos 1; Milestone, Bergamo, Italy) and treated with a warm-up program of 20 min at 1,000 W of microwave power. After the cooling time, the digested samples were diluted to a final volume of 25 ml with ultrapure water (resistivity 18.2 $M\Omega$/cm) obtained from a Milli-Q Integral 3 device (Merck Millipore, Merck KGaA, Darmstadt, Germany). Samples of Mussel Tissue (CE278k) and Bovine Muscle (BB184), both from ERM (European Reference Material, Geel, Belgium), were used to verify the accuracy of the analytical procedures described above. All the solutions were stored in high-density PE bottles cleaned with a 10% (v/v) solution of HNO_3, and were sonicated and rinsed afterward with ultrapure water. For the analysis of minerals, an ICP-OES instrument, Avio200, equipped with a vertical DualView optical system coupled with an S10 autosampler was used. Lengths of the analytical lines (nm) utilized for the analyses are reported in **Table 2**; the Argon line at 420.069 nm was applied as an internal standard. **Table 3** reports the operational conditions of the ICP-OES. Data acquisition and processing were performed using the PerkinElmer Syngistix™ for ICP software (Perkin Elmer, Waltham, MA, United States).

Optical optimization of the ICP-OES was conducted with the procedure of the Syngistix™ software, and torch position

TABLE 3 | Operational conditions of the ICP-OES.

Parameter	Conditions
Radiofrequency power (W)	1,500
Plasma gas flow (L/min)	10.0
Auxiliary gas (L/min)	0.2
Nebulizer gas (L/min)	0.7
Sample uptake (mL/min)	1.0

was optimized before the analytical step using an Mn analytical line with a 1 mg/l Mn solution. The quantification of each mineral was made with external calibration curves using a set of solutions of 0.05, 0.25, 0.5, and 1 ppm arranged from a Perkin Elmer multi-element analytical solution for ICP analysis. The calibration curves for all elements were established using a calibration blank and a reagent blank, and all were found to have correlation coefficients (r^2) ranging from 0.998 to 0.999. Detection limits (DLs) were determined by analyzing a matrix blank represented by reagents and quantities same as those used for sample preparation. Recoveries from the certified materials have reached acceptable values and higher than 85% for most of the elements and up to 95% for Zn and Cu.

Analysis of Microbiological Profile of Fish Muscle

The analyses were carried out on samples of skin dissected from the tail to the opercula, on dorsal fleshes, and on the intestine dissected from the pylorus to the anal opening. Sampling was performed with sterile scissors and forceps by collecting the skin first from one side and the underlying flesh portion, and then repeating the operation on the opposite side and finally sampling the intestine. Each sample of skin, dorsal fleshes, and intestine was split into two aliquots and subjected to microbiological analysis. The aliquots for each sample of skin, dorsal flesh, and intestine were homogenized with buffered peptone water (Biolife, Milano, Italy, at a ratio of 1:9 w/v and with a stomacher (400 Circulator; International PBI s.p.a., Milano, Italy) for 60 s at 230 rpm and tested for the following parameters: (i) enumeration of *Enterobacteriaceae* (45) on Violet Red Bile Glucose Agar (Biolife, Milano, Italy), incubated at 37 ± 1°C for 24 h; (ii) enumeration of *Clostridium* spp. (46) on Tryptose Sulfite Cycloserine Agar (Biolife, Milano, Italy), incubated at 37 ± 1°C for 24 h under anaerobic conditions; (iii) detection of *Salmonella* spp. (47) on Chromogenic Salmonella Agar (Biolife, Milano, Italy) and Xylose Lysine Deoxycholate Agar (Biolife, Milano, Italy), both incubated at 37 ± 1°C for 24 h; (iv) detection and enumeration of *Pseudomonas* spp. on Pseudomonas Agar Base (HiMedia Laboratories, Mumbai, India), incubated at 25 ± 1 °C for 48 h; (v) detection and enumeration of *Aeromonas* spp. on GSP Agar (Pseudomonas Aeromonas Selective Agar Base) acc. to KIELWEIN (Merck, Darmstadt, Germany), incubated at 30 ± 1°C for 48 h; (vi) detection and enumeration of *Vibrio* spp. (48) on TCBS (thiosulfate citrate bile sucrose agar;bioMerieux, Marcy l'Etoile, France), incubated at 37 ± 1°C for 24 h; (vii) detection and enumeration of Specific Spoilage Organisms (SSOs) (49) on Iron Agar (Lyngby) (Oxoid Ltd., Basingstoke, Hampshire, England), incubated at 20 ± 1°C for 72 h counting black colonies as sulfide producers and white colonies as sulfide non-producers. The LODs were 10 CFU/g for the count of *Enterobacteriaceae*, *Aeromonas* spp., *Clostridium* spp. and SSOs, and 100 CFU/g for the count of *Pseudomonas* spp. The other aliquots for each sample of skin, dorsal flesh, and intestine were processed for the detection of *Listeria monocytogenes* (50) as follows: they were homogenized with Listeria Fraser Broth Half Concentration (Biolife, Milano, Italy), incubated at 30 ± 1°C for 20 h, followed by a passage in Listeria Fraser Broth (Biolife, Milano, Italy) incubated at 37 ± 1°C for 24 h and spread both on Agar Listeria according to Ottaviani and Agosti (Biolife, Milano, Italy) and Listeria Palcam Agar (Biolife, Milano, Italy), both incubated at 37 ± 1°C for 24–48 h.

Statistical Analysis

For chemical composition of the filets, all the data were analyzed with the ANCOVA procedure of the XLSTAT statistical package (51). The diets (HIM0, HIM25, HIM35, and HIM50%) were used as a fixed effect and the final body weight as the covariate. In this way, the possible effects of diet and body weight have been separated. Separation of means was assessed by Tukey's test, and differences were significant if $p < 0.05$.

To evaluate the influence of the different dietary formulations on the microbiological profile of the skin, intestine, and muscle of the fish, the microbial loads of each parameter between the different groups were compared. The normal distribution of the raw data was tested by a D'Agostino-Pearson omnibus test, and a one-way analysis of variance (ANOVA) was conducted to evaluate any significant differences between each group. A *post hoc* Tukey's test was conducted for the multiple comparisons in the obtained ANOVA data. Critical significance level (p) was set at 5% (0.05), and all the tests were performed two-sided. All the statistical analyses were carried out with the Graph Pad Prism 9 software (San Diego, CA, United States).

RESULTS

Fish Growth Performance

In brief, at the end of the feeding trial, all the fish almost tripled their mean body weight, but there were no significant differences ($p > 0.05$) between the dietary groups for any of the considered growth performance and feed utilization efficiency indices.

Chemical Composition of Filets

Table 4 reports the chemical composition of sea bream filet muscle. Moisture, crude protein, total lipids, and ash contents of the filets were not affected by the dietary treatments.

The fatty acid composition of the sea bream filet muscle is shown in **Table 5**. The saturated fatty acid, the lauric acid (C12:0) and the myristic acid (C14:0) showed significantly higher values in the HIM50% group than those observed in the fish fed fish meal and lower inclusions of *Hermetia illucens* meal. The unsaturated fatty acids, myristoleic acid (C14:1), alpha-linolenic acid (ALA, C18:2n6), and arachidonic acid (ARA, C20:4n6) showed significantly higher values in the HIM50%

TABLE 4 | Proximate composition (g/100 g of wet weight) of the filets of the gilthead sea bream fed with the four exerimental diets.

	GROUP				*p*-value		SEM[1]
	HIM0	HIM25%	HIM35%	HIM50%	D[2]	BW[3]	
Fish	18	18	18	18			
Moisture	67.11	67.24	66.79	67.68	0.354	0.110	0.287
Crude Protein	20.41	19.99	20.07	19.82	0.242	0.824	0.183
Total Lipids	10.78	11.13	11.48	10.83	0.273	0.304	0.275
Ash	1.70	1.64	1.66	1.67	0.869	0.392	0.049

HIM0, fish meal group; HIM25, HIM35, and HIM50%, Hermetia illucens meal at 25, 35, and 50% substitution rates of fish meal groups, respectively.
Fish: 18 per each diet, 6 fish per tank, and 3 replications per diet.
[1]*Standard error of the mean.*
[2]*Diet.*
[3]*Body weight.*

group than those observed in the fish fed with fish meal and lower inclusions of the *Hermetia illucens* meal; oleic acid (C18:1n9) showed a significantly higher level in the HIM25 and HIM35% groups than in the HIM50% group; eicosapentaenoic acid (EPA, C20:5n3) showed a similar content in the HIM0, HIM35 and HIM50% groups, which was significantly higher than that in the HIM25% group; docosahexaenoic acid (DHA, C22:6n3) showed a significantly higher content in the fish fed with fish meal than in the fish fed with the insect meal. The fatty acid classes of the filets are reported in **Table 6**. The sum of the saturated fatty acids (SFAs) showed a significantly higher value in the HIM50% group than the observed value in the HIM0 group. The sum of the monounsaturated fatty acids (MUFAs) showed a significantly lower value in the HIM50% group than the observed in the fish with fed fish meal and lower inclusions of the *Hermetia illucens* meal. The sum of the polyunsaturated fatty acids (PUFAs) and PUFAs of the n3-series showed a similar content in the HIM0, HIM35, and HIM50% groups, which was higher than that in the HIM25% group, whereas the fatty acids of the n6-series showed a significantly higher value in the HIM50% group than the observed in the fish fed with fish meal and with lower levels of inclusion of the insect meal. n3/n6 PUFA ratio (**Table 6**), as well as the sum of EPA+DHA, showed significantly higher levels in the fish with fed with fish meal than those of the fish fed with different inclusion of the insect meal. The indices of nutritional interest, i.e., the atherogenic (AI), thrombogenic (TI), and peroxidation (PI) indices and hypocholesterolemic/hypercholesterolemic (H/H) ratio are reported in **Table 6**. No significant ($p > 0.05$) difference was observed for TI and H/H in the groups, whereas the AI showed a similar level in the HIM0, HIM25, and HIM35% groups but was significantly lower and, therefore, better than that recorded in the HIM50% group. On the contrary, the PI showed a similar content in the HIM0, HIM35, and HIM50% groups but was higher than that in the HIM25% group. Body weight did not significantly ($p > 0.05$) affect the fatty acid classes, the ratio, or the nutritional indices.

Twenty amino acids, ten indispensable amino acids (EAA), and ten dispensable ones (NEAA), were identified and quantified in the sea bream filet muscle (**Table 7**). Among the indispensable amino acids in the filets, arginine and phenylalanine showed significantly higher values in fish fed with fish meal than in fish fed with different inclusion of the insect meal; isoleucine and valine showed significantly higher values in fish fed with higher inclusion of the insect meal (HIM50%) than in fish fed with fish meal and with lower levels of inclusion of the insect meal; leucine, lysine and methionine showed significantly lower levels in fish of the HIM35% group, histidine in fish of the HIM25% group, and tryptophan in fish of the HIM50% group than those in the other groups. Threonine showed similar values ($p > 0.05$) in the groups. Among the dispensable amino acids, alanine showed a significantly higher level in filets of the HIM50% group than those observed in the HIM25 and HIM35% groups; aspartate + asparagine showed a significantly higher level in filets of the HIM50% group than that observed in the filets of fish fed with fish meal; glycine showed a significantly higher level in filets of the HIM50% group than that of HIM35% group; cysteine and tyrosine showed significantly higher levels in the filets of fish fed with fish meal than those of the fish fed with different inclusion of the insect meal. Hydroxylysine, hydroxyproline, and serine showed similar values in the groups. The EAA/NEAA ratio in the filets showed a significantly higher value in the fish fed with fish meal than in the fish fed with different inclusion of the insect meal. Body weight did not significantly ($p > 0.05$) affect the indispensable or the dispensable amino acid profile.

Table 8 shows the average mineral content values in the sea bream filet muscle: macrominerals (phosphorus, sodium, potassium, calcium, and magnesium), microminerals (zinc, iron, manganese, copper, and chromium), and trace minerals (nickel). Phosphorus, showed a significantly higher value in the fish of the HIM25% group than that observed in the HIM0, HIM35, and HIM50% groups. Calcium showed a significantly higher value in the filets of the HIM0 group than those observed in the HIM50% group, whereas the HIM25 and HIM35% groups showed intermediate values. Sodium, potassium, and magnesium showed similar values ($p > 0.05$) in the groups. Due to the antagonist interaction of the Ca and P, the concentration ratio between these macroelements was calculated (52). A significantly higher level of the Ca/P ratio was observed in the HIM0 group than in the fish fed with different inclusions of *Hermetia illucens*.

TABLE 5 | Fatty acid composition (g/100 g of fatty acid methyl esters)# of the filets of the gilthead sea bream fed with the four exerimental diets.

	GROUP				*p*-value		SEM[1]
	HIM0	HIM25%	HIM35%	HIM50%	D[2]	BW[3]	
Fish	18	18	18	18			
C12:0	0.05 d	0.23 c	0.40 b	0.58 a	<0.0001	0.265	0.001
C14:0	2.57 b	2.59 b	2.63 ab	2.75 a	0.001	0.636	0.012
C14:1	0.06 b	0.06 b	0.06 b	0.07 a	0.001	0.320	<0.0001
C15:0	0.20 a	0.19 b	0.19 b	0.19 b	0.001	0.690	<0.0001
C16:0	15.29	15.31	15.12	15.41	0.626	0.944	0.257
C16:1	4.17	4.16	4.25	4.16	0.610	0.716	0.031
C17:0	0.17 a	0.16 b	0.16 b	0.17 a	0.003	0.610	<0.0001
C17:1	0.03	0.03	0.03	0.03	0.296	0.407	<0.0001
C18:0	3.41	3.45	3.40	3.38	0.898	0.113	0.054
C18:1n9	40.62 ab	41.11 a	40.78 a	40.02 b	<0.0001	0.715	0.308
C18:1n7	3.11 a	3.15 a	3.06 ab	3.01 b	0.001	0.996	0.062
C18:2n6	12.83 c	12.78 c	13.17 b	13.52 a	<0.0001	0.231	0.062
C18:3n6	0.17	0.16	0.18	0.15	0.058	0.031	0.001
C18:3n3	3.26	3.28	3.25	3.30	0.661	0.348	0.010
C20:0	0.25	0.26	0.25	0.26	0.352	0.770	<0.0001
C20:1n9	2.02 a	2.02 a	1.82 b	1.79 b	<0.0001	0.339	0.006
C20:2n6	0.29 b	0.29 b	0.31 b	0.33 a	<0.0001	0.460	0.001
C20:3n6	0.16	0.17	0.17	0.17	0.505	0.112	<0.0001
C20:4n3	0.39 a	0.35 b	0.37 ab	0.36 b	0.0003	0.682	0.001
C20:4n6 ARA	0.12 b	0.12 b	0.13 ab	0.14 a	0.0002	0.224	<0.0001
C20:5n3 EPA	3.41 a	3.20 b	3.33 a	3.32 a	0.0002	0.026	0.012
C22:0	0.14	0.14	0.14	0.15	0.675	0.823	<0.0001
C22:1n9	1.12 a	0.99 b	0.93 b	0.94 b	0.0005	0.738	0.012
C22:5n3 DPA	1.28 b	1.27 b	1.34 a	1.34 a	0.047	0.677	0.006
C22:6n3 DHA	4.80 a	4.48 b	4.46 b	4.41 b	0.008	0.588	0.077

HIM0, fish meal group; HIM25, HIM35, and HIM50%, Hermetia illucens meal at 2, 35, and 50% substitution rates of fish meal groups, respectively.
Fish: 18 per diet, 6 fish per tank, and 3 replications per diet.
#Concentration of fatty acid is expressed as g/100 g, considering 100 g the sum of the areas of all the fatty acid methyl esters (FAMEs) identified.
Mean values with different letters in the same row are significantly different at $p < 0.05$.
ARA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[1]Standard error of the mean.
[2]Diet.
[3]Body weight.

Among the microminerals, manganese, copper, and chromium showed similar values ($p > 0.05$) in the groups. Zinc showed a significantly lower value in fish of the HIM25 and HIM50% groups than in those of the HIM0 and HIM35% groups. Iron was significantly higher ($p = 0.011$) in the filets of the HIM0 group than in those of HIM50%, whereas intermediate values in the HIM25% and HIM35% groups were observed. The only trace mineral identified was nickel, which showed a significantly higher ($p = 0.044$) value in fish of the HIM25% group than in those of the HIM35% group; intermediate values in fish of the HIM0 and HIM50% groups were observed. Body weight did not significantly ($p > 0.05$) affect the mineral profile or the Ca/P ratio.

Microbiological Profile of Filets

The results of the microbial analysis are summarized in **Table 9**. In the dorsal flesh of the fish from all the tested groups, no colony of the researched microorganisms was detected. No *Clostridium* spp., *Salmonella* spp., *Aeromonas*spp., *Vibrio* spp., and *L. monocytogenes* were detected in the skin and intestine of the tested specimens. The *Enterobacteriaceae* loads detected in the intestine of fish of all the groups were <2 log cfu/g, while they were not detected in the skin of any fish. SSO loads detected in the skin and intestine were <2 log cfu/g in the HIM0 and HIM25% groups but slightly higher than 2 Log cfu/g in the HIM35 and HIM50% groups although did not reach statistical significance. Regarding SSOs in the samples of the HIM0% group, only white colonies were detected while, in the samples of the HIM25, HIM35, and HIM50% groups, the white colonies compared to the black colonies accounted for majority (> 98%). *Pseudomonas* spp. was detected in the skin and intestine of all the specimens tested (≤2 log cfu/g on average). Significantly ($p < 0.05$) higher loads were observed for the

TABLE 6 | Fatty acid classes and nutritional indices of the filets of the gilthead sea bream fed with the four exerimental diets.

	GROUP				*p*-value		SEM[1]
	HIM0	HIM25%	HIM35%	HIM50%	D[2]	BW[3]	
Fish	18	18	18	18			
SFA	22.08 b	22.33 ab	22.28 ab	22.88 a	0.042	0.619	0.469
MUFA	51.14 a	51.51 a	50.93 a	50.00 b	<0.0001	0.792	0.360
PUFA	26.72 a	26.10 b	26.72 a	27.05 a	<0.0001	0.318	0.224
n3	13.15 a	12.57 b	12.75 ab	12.74 ab	0.003	0.512	0.134
n6	13.57 c	13.52 c	13.97 b	14.31 a	<0.0001	0.380	0.071
n3/n6	0.97 a	0.93 b	0.91 bc	0.89 c	<0.0001	0.995	0.001
EPA+DHA	8.21 a	7.68 b	7.79 b	7.73 b	0.001	0.747	0.100
AI	0.33 b	0.33 b	0.34 b	0.35 a	0.001	0.941	<0.0001
TI	0.29	0.30	0.30	0.30	0.182	0.501	<0.0001
H/H ratio	3.72	3.70	3.75	3.64	0.379	0.964	0.023
PI	64.62 a	61.95 b	63.17 ab	63.30 ab	0.004	0.651	2.910

HIM0, fish meal group; HIM25, HIM35, and HIM50%, Hermetia illucens meal at 25, 35, and 50% substitution rates of fish meal groups, respectively.
Fish: 18 per diet, 6 fish per tank, and 3 replications per diet.
SFA, sum of the saturated fatty acids; MUFA, sum of the monounsaturated fatty acids; PUFA, sum of the polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AI, atherogenic Index; TI, thrombogenic Index; PI, peroxidation index; H/H, hypocholesterolaemic/hypercholesterolaemic ratio. Mean values with different letters in the same row are significantly different at $p < 0.05$.
[1] *Standard error of the mean.*
[2] *Diet.*
[3] *Body weight.*

Pseudomonas spp. in the skin of the HIM50% group compared to the HIM25% group.

DISCUSSION

The diet containing insect meal did not affect the chemical composition of sea bream (*Sparus aurata*) muscle. A slight decrease in protein content was observed in fish fed with insect meal, although this variation was not statistically significant. The data are in accordance with studies on Atlantic salmons fed with a larva meal from *Hermetia illucens* (9) and sea breams fed with a larva meal from *Tenebrio molitor* (53).

Among the fatty acids in fish filets, the most interesting results were regarding the significant differences observed for some polyunsaturated fatty acids of nutritional interest. These observations are not consistent with the dogma in that differences in the fatty acid composition of muscle lipids reflect differences in dietary fatty acid contents (53, 54). In fact, despite the different *Hermetia illucens* inclusions into the diet (33) not being able to modify the fatty acid content of the feeds, the fatty acids in the sea bream muscles showed a different trend. Moreover, as observed by Sealey et al. (54), some muscle fatty acid concentrations were attenuated relative to dietary content. Among these, oleic acid (C18:1n9) ranged from 43 to 45% in the diets (33), but it only ranged from 40 to 41% in the muscles, and alfa-linolenic acid (C18:3n3) ranged from 4 to 5% in the diets (33) but was only about 3% in the muscles of all the groups. A higher concentration of DHA (C22:6n3) was detected in the muscle (4.54% on average) in comparison to its content in the diets (3.61% on average). Regarding the trend of DHA content in the muscle, significantly lower values were found in fish fed with insect meal than in those fed with the basal diet (100% fish meal), although the diets contained similar levels of DHA [Oteri et al. (33)] and were formulated to provide DHA above the estimated EFA requirements (55). The results are in agreement with the observations of Belforti et al. (8) and Pulido et al. (56). EPA appeared reduced in muscle fatty acids (3.32%, on average) compared to dietary concentrations (4.75%, on average) (33). The results agree with Sealey et al.'s observations (54), but they are not in agreement with St-Hilaire et al. (26) and Ewald et al. (28) who observed a decrease in ALA and EPA in fish fed with the inclusion of HIM in the feed. It is assumed that marine fish have a deficient capacity to bioconvert 18C precursors (C18: 2n6 and C18: 3n3) into LC-PUFAs with 20 or 22 carbon atoms such ARA, EPA, and DHA, thanks to the activity of Δ6, Δ5, and Δ4 fatty acid desaturases (57, 58). For this reason, marine fish require the presence of preformed LC-PUFAs in the diet. However, Seiliez et al. (59) demonstrated the presence and nutritional modulation of a Δ6 fatty acid desaturase in *Sparus aurata*. The same authors did not detect Δ5 and Δ4 fatty acid desaturases; the latter are responsible for the synthesis of DHA from DPA (C22: 5n3). Therefore, clear explanation and interpretation of the results obtained appear difficult, as the metabolic pathway of long-chain polyunsaturated fatty acids (LC-PUFAs) in marine fish is still under debate.

It must be mentioned tha tEPA and DHA are essential for the growth, development and health, and regulation of expression of several genes involved in lipid metabolism (60). ARA and EPA play a major role in eicosanoid production (61). The results showed that in the filets of all the experimental groups, the

TABLE 7 | Amino acid composition (g/100 g of wet weight) of the filets of the gilthead sea bream fed with the four exerimental diets.

	GROUP				*p*-value		SEM[1]
	HIM0	HIM25%	HIM35%	HIM50%	D[2]	BW[3]	
Fish	18	18	18	18			
Indispensable amino acids							
Arginine	1.77 a	1.11 b	1.05 c	1.16 b	<0.001	0.295	0.014
Histidine	0.83 a	0.77 b	0.82 ab	0.86 a	<0.001	0.701	0.013
Isoleucine	1.23 b	1.25 b	1.20 b	1.34 a	<0.0001	0.404	0.014
Leucine	2.12 a	2.07 a	1.93 b	2.11 a	<0.001	0.463	0.018
Lysine	3.66 a	3.65 a	3.44 b	3.55 ab	0.011	0.236	0.047
Methionine	0.66 a	0.59 b	0.56 c	0.66 a	<0.001	0.884	0.008
Phenylalanine	1.56 a	1.16 c	1.34 b	1.04 d	<0.001	0.626	0.011
Threonine	1.10	1.18	1.16	1.16	0.212	0.691	0.027
Valine	1.15 b	1.17 b	1.13 b	1.26 a	<0.001	0.705	0.015
Tryptophan	0.02 b	0.03 a	0.03 a	0.02 c	<0.001	0.855	0.011
Dispensable amino acids							
Hydroxylysine	0.14	0.12	0.13	0.12	0.630	0.276	0.009
Alanine	0.96 ab	0.95 b	0.96 b	1.00 a	0.013	0.187	0.011
Aspartate+Asparigine	1.39 b	1.41 ab	1.39 ab	1.48 a	0.032	0.841	0.024
Cysteine	0.06 a	0.03 b	0.04 b	0.01 c	<0.001	0.766	0.003
Glycine	0.93 ab	0.91 ab	0.85 b	0.95 a	0.007	0.478	0.019
Glutamate+ Glutammine	0.77	0.74	0.76	0.75	0.823	0.598	0.021
Proline	0.73 a	0.72 ab	0.68 b	0.76 a	0.001	0.666	0.013
Hydroxyproline	0.33	0.33	0.34	0.34	0.456	0.193	0.008
Tyrosine	1.03 a	0.97 b	0.90 c	0.91 c	<0.001	0.889	0.014
Serine	1.07	1.15	1.14	1.09	0.058	0.615	0.024
EAA/NEAA	1.91 a	1.77 b	1.76 b	1.78 b	<0.001	0.206	0.018

HIM0, fish meal group; HIM25„ HIM35, and HIM50%, Hermetia illucens meal at 25, 35 and 50% substitution rates of fish meal groups, respectively.
Fish: 18 per diet, 6 fish per tank, and 3 replications per diet.
Mean values with different letters in the same row are significantly different at $p < 0.05$.
[1]Standard error of the mean.
[2]Diet.
[3]Body weight.

ARA/EPA ratio did not change (0.04), and that the levels of DHA were more depressed than those of EPA, as indicated by the changes in the EPA/DHA ratio (from 0.71 to 0.75). Moreover, Pulido et al. (56), with the aim of evaluating to what extent replacing fish meal with insect meal could alter not only the fatty acid (FA) profile of filets of *Sparus aurata* but also the FA distribution inside tryglicerides, observed that the inclusion of HIM reduced n3-PUFAs in sea bream filets but did not substantially change the presence of fatty acids important for human nutrition (e.g., EPA and DHA) in the *sn*-2 position of filet triglycerides, increasing the chances of being better assimilated and absorbed by potential consumers. The values recorded for some health lipid indices (TI: thrombogenic index and H/H: hypocholesterolemic/hypercholesterolemic ratio) appeared to be of interest, into account the contribution that each fatty acid has to influence the incidence of cardiovascular diseases (40). The similar values of these indices in the filets of all the groups suggest similar nutritional effects of all the diets on the animals. This result could be due to chitin, the main component of the exoskeleton of insects. Chitin contains high levels of chitosan with cholesterol-lowering properties in fish (62, 63), it binds with lipid micelles (cholesterol), inhibits their absorption, and increases the excretion of bile acid; thus, it interferes with the absorption of cholesterol (64). As observed by Iaconisi et al. (53), the atherogenic index showed the worst value in filets of fish fed with highest inclusion of HIM (HIM50% group), testifying a greater probability of fatty acids to affect the incidence of cardiovascular diseases (40). However, the AI and TI values observed in filets appeared much lower and, therefore, better than those reported in terrestrial animal foods (8, 65). The peroxidation index considers the contribution that PUFAs make in influencing oxidative degradation. The control of this process, causing loss of nutritional value and formation of anti-nutritional molecules, can play a central role in maintaining muscle quality (66). Lastly, the highest level of lauric acid observed in fish filets of the HIM50% group, did not affect ($p > 0.05$) fish growth performance (final body weight: 390 ± 49 g; specific growth rate: 0.75 ± 0.02). This fatty acid is dominant in black soldier fly larvae (28, 32) and is considered a bioactive compound for a possible role as an antimicrobial counteracting antibiotic resistance (67).

TABLE 8 | Mineral element profile (mg/kg of wet weight) of the filets of the gilthead sea bream fed with the four exerimental diets.

	GROUP				*p*-value		SEM[1]
	HIM0	HIM25%	HIM35%	HIM50%	D[2]	BW[3]	
Fish	18	18	18	18			
Macrominerals							
P - Phosphorus	2,356 c	12,016 a	9,459 b	8,880 b	<0.0001	0.575	300.323
Na-Sodium	665	578	520	6340	0.254	0.652	53.05
K-Potassium	3286	3414	3503	3462	0.453	0.493	98.81
Ca-Calcium	943 a	625 ab	590 ab	484 b	0.019	0.484	96.01
Mg-Magnesium	358	344	367	356	0.709	0.526	13.83
Ca/P ratio	0.41 a	0.05 b	0.07 b	0.05 b	<0.0001	0.088	0.03
Microminerals							
Zn-Zinc	24.96 a	17.92 b	22.36 a	16.44 b	<0.0001	0.466	2.14
Fe-Iron	5.73 a	4.09 ab	4.47 ab	3.60 b	0.011	0.281	0.41
Mn-Manganese	0.41	0.50	0.49	0.43	0.635	0.080	0.06
Cu-Copper	0.31	0.35	0.52	0.35	0.110	0.693	0.06
Cr-Chromium	0.37	0.41	0.46	0.48	0.156	0.124	0.05
Se-Selenium	0.30 a	0.22 b	0.22 b	0.35 a	<0.0001	0.377	0.02
Trace mineral							
Ni-Nickel	1.13 ab	1.17 a	0.75 b	1.10 ab	0.044	0.094	0.101

HIM0, fish meal group; HIM25, HIM35, and HIM50%, Hermetia illucens meal at 25, 35, and 50% substitution rates of fish meal groups, respectively.
Fish: 18 per diet, 6 fish per tank, and 3 replications per diet.
Mean values with different letters in the same row are significantly different at $p < 0.05$.
[1] *Standard error of the mean.*
[2] *Diet.*
[3] *Body weight.*

TABLE 9 | Microbial profile (log cfu/g) of the skin, intestine, and muscle of the gilthead sea bream fed with the four exerimental diets.

Items	GROUP											
	HIM0%			HIM25%			HIM35%			HIM50%		
	Skin	Intestine	Muscle	Skin	Intestine	Muscle	Skin	Intestine	Muscle	Skin	Intestine	Muscle
Enterobatteriaceae	<1	1.76	<1	<1	1.83	<1	<1	1.83	<1	<1	1.83	<1
SSOs[1]	1.90	1.93	<1	1.86	1.96	<1	2.03	2.04	<1	2.04	2.04	<1
Pseudomonas spp.	1.66ab	1.67	<1	1.68b	1.82	<1	1.91ab	1.87	<1	1.95a	1.93	<1
Aeromonas spp.	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Vibrio spp.	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Clostridium spp.	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
L. monocytogenes	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella spp.	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

[1] *Specific spoilage organisms.*
Data reported are expressed as mean values of 9 samples analyzed (3 fish per tank and 3 replications per diet).
Mean values with different letters in the same row are significantly different at $p < 0.05$.

Based on our knowledge, this study is the first to test the effects of *Hermetia illucens* meal dietary inclusion on the amino acid composition of sea bream filets, so few comparisons with the literature are possible. On the whole, the essential amino acid profile of the fish filets reflected high protein quality. As observed by Iaconisi et al. (68), Lys and Leu were the most representative EAAs in fish filets and are the same EAAs contained at a high level in the corresponding feeds (33). Quantitatively, their content in fish filets was similar in the groups, with the exception of the muscles of the HIM35% group, although the Lys and Leu content was higher in feeds containing insect meal as a partial substitute for fish meal. One possible explanation could be that, although all the diets were formulated to be isonitrogenous, the chitin content of the feeds containing insect meal may have reduced the levels of digestible proteins or, more specifically, available amino acids (69). In fact, chitin, an unbranched N-acetylglucosamine polymer, is indigestible for many fish species that are devoid of chitinase activity (70) or with limited activity (18, 71–73). This leads to impaired digestibility of other nutrients with consequent increase in bulk, reduced feces retention time, and reduced enzyme accessibility to substrates (74). Nonetheless, the growth performance of the fish (final body weight: 390 ± 49 g; specific growth rate:.75 ± 0.02) was similar in the groups and was not affected by the dietary incorporation of HIM. Furthermore, as reported by Belghit et al. (9), *Hermetia illucens* larvae have a well-balanced EAA profile, with the exception of lysine, methionine, and tryptophan, close to that of the fish meal, considered as the protein with the best EAA profile for fish (75). In this trial, in relation to the levels of FM replacement by HIM, these amino acids were added to the diets to meet the needs of *Sparus aurata* as suggested by Magalhães et al. (76). As quantification of EAA requirements is generally based on analysis of dose-response curves with weight gain used as response criterion (77), the similar results obtained for the *in vivo* performance of the fish of all groups (final body weight: 390 ± 49 g; specific growth rate:.75 ± 0.02) demonstrate that all the diets meet the dietary amino acid requirement of *Sparus aurata*. NEAAs are not strictly necessary in the diet, because fish can synthesize them on their own; however, NEAAs can have beneficial effects on fish health and performance when present in the right concentration (78). In this study, although the diets containing the insect meal had a higher NEAA level (33) than the diet containing exclusively fish meal, the NEAA content in fish filets of all the groups was similar. This result is not in accordance with the observations of Belghit et al. (9) and could be due, as reported above, to the dietary content of chitin.

Minerals are divided into macroelements, whose needs by organisms are in large quantities, microelements, whose needs by organisms are in small quantities (79), and trace minerals typically required by organisms in such small quantities that a dietary supplement is not required (77). The functions of macrominerals include formation of skeletal structures and other hard tissues, electron transfer, regulation of acid: base equilibrium, production of membrane potentials, and osmoregulations (77). Among microelements, calcium and phosphorus are two of the major constituents of the inorganic portions of diets for fish. Quantitatively, calcium and phosphorus function primarily as structural components of hard tissues. Dietary deficiencies of most macrominerals such as calcium have been generally difficult to produce with fish species because of the presence of these ions in water (77). On the contrary, concentrations of phosphorus in natural waters are generally very low (77). Deficiency of dietary phosphorus impairs intermediate metabolism and causes reduction in fish growth and feed conversion. Integrating phosphorus into fish diets is generally more critical, because its presence in water and use by fish are limited. However, the influence of excreted phosphorus on eutrophication of receiving waters has led to a significant amount of research focused on phosphorus nutrition with the aim of minimizing phosphorus excretion (77). This appears to be of particular interest in relation to the results obtained in this study, where the phosphorus content was significantly lower in the fish filets of the control group (HIM0 = 2.4 g/kg) fed with diet with highest phosphorus content (11.45 g/kg) and formulated exclusively with fish meal (33). As reported by Rodehutscord and Pfeffer (80), phosphorus concentrations in practical dietary formulations mainly based on fish meal considerably exceed the estimation requirements. Therefore, excess in dietary phosphorus and low amount of absorbed phosphorus by fish lead to a problem of environmental impact caused by surplus phosphorus discharge into the effluents (81). However, muscle tissuess are not considered to be specific physiological sites for calcium and phosphorus (82). Phosphorus and calcium accumulate in largest amounts in bones. Borucka-JastrzeBska et al. (83) determined micro- and macroelement concentrations in different tissues of fish, and they reported that calcium distribution followed the same pattern for all three analyzed species in decreasing order: gills > muscles > skin > liver > kidney > blood. Perkowska and Protasowicki (84) showed that high levels of heavy metals were in the liver, and that the lowest ones were in the muscles. Moreover, in fish species analyzed by Roméo et al. (85), the content of metals was higher in the gills than in the muscles. Gills and liver are chosen as target organs for assessing metal accumulation. Therefore, the significantly lower values of the Ca/P ratios in fish receiving insect meal should not cause a concern. In fact, the Ca e P content did not affect anomalies in mineral homeostasis and bone mass (86). Heavy metals in the marine environment and fish contamination not only pose a threat to fish health, but by accumulating as they flow down to the natural food chain, they also pose a risk to human health (87, 88). Therefore, it is necessary to determine their content in widely consumed fish species such as *Sparus aurata*. Heavy metals such as manganese, iron, cobalt and copper are necessary for fish metabolism (89) but are toxic at high concentrations (90), while cadmium, chromium, mercury, lead and nickel are toxic metals even if present in traces in both humans and animals (91) causing numerous damages to organs (92). Although chromium is a ubiquitous metal in the environment and trivalent chromium is essential for biolife, hexavalent chromium is said to be a toxic metal with mutagenic, carcinogenic, and harmful impacts on the biota. Researchers revealed that chromium affects the physiological, behavioral, histological, biochemical, genetic, and immunological conditions of experimental organism (93). The chromium concentrations in the fish filets were found to be below the permitted level set by the European Union at 0.5 mg/kg wet weight (94). Manganese functions as a cofactor in several enzyme systems, including those involved in urea synthesis from ammonia, amino acid metabolism, fatty acid metabolism, and glucose oxidation (95). Manganese levels in fish filets were found to be below the permitted levels established by the FAO/WHO (96). Iron, essential in fish as a heme protein compound (e.g., hemoglobin, myoglobin, and cytochromes) or as a nonheme protein compound (e.g., transferrin, ferritin, and hemosiderin) (79) and for its involvement in cellular respiration both in oxidation-reduction and electron transport activity (97), also showed values below the maximum levels set by the FAO/WHO (96). Copper is an important micromineral in fish metabolism and is important for hemoglobin synthesis in many enzymatic reactions (98), but high copper concentrations can cause liver and kidney damages (99). The copper concentration determined in the fish filets of this study was below the values set by the standard regulatory limits allowed in fish samples (96). Nickel is an environmental factor that occurs at a very low level and can cause serious lung health problems such as lung cancer, fibrosis, emphysema, cancer, and kidney disease (100). In this study, nickel values were considerably lower than the permitted levels set by the FAO/WHO (96). Finally, zinc, involved in various metabolic pathways such as protein synthesis, growth, immunity, and energy metabolism in fish (79, 97) showed a significantly lower level in the fish fed with an inclusion of 25 and 50% of HIM. Overall, considering that several studies indicated that the content of these microelements in fish is influenced by surrounding water (77, 101) and by food source (102), which is the major source of elements such as iron, zinc, manganese and copper, the data would seem to highlight that the inclusion of HIM into the three diets maintains lower heavy metal levels than those recommended by various authorities (FAO, WHO, and EU).

Despite the observed differences in the lipid, protein, and mineral profiles of the filets, the organoleptic properties, in terms of color, volatile fraction, and taste, of the 4 groups of fresh filets resulted similar between the groups, suggesting that the use of HIM does not alter significantly the organoleptic properties of *Spaurus aurata* filets (34).

With regard to biological hazards, the EFSA opinion identifies the substrate used to feed the insects as the key entrance point for contamination (15); therefore, pathogenic bacteria may be present in insects depending on the substrate used and rearing conditions. Mucosal tissues, including skin and gut, are in direct contact with the environment and, thus, are the first contact points of microbes with their host representing a good control point for fish health and consumer safety (103). The reported results are confirmed, as the insect meal incorporation into the diets did not significantly influence the microbiological profile of the fish. *Pseudomonas* spp. belongs to the group of the SSOs whose loads did not differ between the various groups; therefore, although the loads of *Pseudomonas* spp. in the skin of the HIM50% group was significantly higher than in HIM25%, the detected values do not represent a relevant risk to public health. The feed can impact the microbial quality of the fish both directly, if microbiologically poor, and indirectly if residing, due to inadequate breeding set up and management, leads to modifications of water parameters (104). Therefore, we could speculate that the very low loads observed in this study are related to the good microbial quality of the feeds used, characterized only by a few SSOs with loads below 1.85 log cfu/g, as previously reported by Oteri et al. (33), as well as to the good conditions of the experimental aquaculture facility. Further studies are desirable to evaluate their application in a real scenario.

CONCLUSIONS

This study indicates that the *Hermetia illucens* meal as a partial substitute for fish meal did not affect the proximate composition of the fish filets but significantly affected the fatty acid and amino acid profiles. However, since no detrimental effects on growth performance were found, the effects on filet quality should be considered. Furthermore, the heavy metal content and microbiological quality of the fish filets underline the safety of the *Hermetia illucens* meal as animal feed. As the price of fishmeal and fish oil increases, an economic analysis of incorporation of the *Hermetia illucens* meal into diets is needed to better assess its role as an affordable and sustainable feed in aquaculture.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Italian Ministry of Health (Ministerial Authorization Number 491/2019-PR released on date 4th July 2019).

AUTHOR CONTRIBUTIONS

MO: formal analysis and writing (original draft preparation and review and editing). BC: conceptualization, methodology, investigation, data curation, writing (original draft preparation and review and editing), supervision, and funding acquisition. GM: investigation and resources. GT: formal analysis. LN and VL: formal analysis and writing (original draft preparation). ADR: methodology, software, data curation, and writing (review and editing). All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Parisi G, Tulli F, Fortina R, Marino R, Bani P, Dalle Zotte A, et al. Protein hunger of the feed sector: the alternatives offered by the plant world. *Ital J Anim Sci.* (2020) 19:1204–25. doi: 10.1080/1828051X.2020.1827993
2. EUMOFA. *Fishmeal and Fish Oil, Maritime Affairs and Fisheries, Production and Trade Flows in the EU*. Brussels: EUMOFA (2021).
3. Tacon AGJ, Hasan MR, Metian M. *Demand and Supply of Feed Ingredients for Farmed Fish and Crustaceans: Trends and Prospects. FAO Fisheries and Aquaculture Technical Paper No 564*. Rome: FAO (2011).
4. Hardy RW. Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquac Res.* (2010) 41:770–6. doi: 10.1111/j.1365-2109.2009.02349.x
5. Turchini GM, Torstensen BE, Ng WK. Fish oil replacement in finfish nutrition. *Rev Aquac.* (2009) 1:10–57. doi: 10.1111/j.1753-5131.2008.01001.x
6. Montero D, Izquierdo M. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. In: Turchini GM, Ng WK, Tocher DR, editors. *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. Boca Raton, FL: CRC Press (2010). p. 439–85.
7. Torrecillas S, Román L, Rivero-Ramírez F, Caballero MJ, Pascual C, Robaina L, et al. Supplementation of arachidonic acid rich oil in Europeanseabassjuveniles (*Dicentrarchuslabrax*) diets: Effects on leucocytes and plasma fatty acid profiles, selected immune parameters and circulatingprostaglandinslevels. *Fish Shellfish Immunol.* (2017) 64:437–45. doi: 10.1016/j.fsi.2017.03.041
8. Belforti M, Gai F, Lussiana C, Renna M, Malfatto V, Rotolo L, et al. The meaning of the sentence is not clear; please rewrite or confirm that the sentence is correct. S, Schiavone A Tenebrio molitor meal in rainbow trout (Oncorhynchus mykiss) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets Ital. *J Anim Sci.* (2015) 14:4170. doi: 10.4081/ijas.2015.4170
9. Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, et al. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture.* (2019) 503:609–19. doi: 10.1016/j.aquaculture.2018.12.032
10. Makkar HP, Tran G, Heuzé V, Ankers P. State-of-the-art on use of insects as animal feed. *Anim Feed Sci Technol.* (2014) 197:1–33. doi: 10.1016/j.anifeedsci.2014.07.008
11. Veldkamp T, Bosch G. Insects: a protein-rich feed ingredient in pig and poultry diets. *AnimFron.* (2015) 5:45–50. doi: 10.2527/af.2015-0019
12. Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi V, et al. Use of *Tenebrio molitor* larvaemealasprotein source in broilerdiet: effect on growth performance, nutrientdigestibility, carcass and meat traits. *J Anim Sci.* (2016) 94:639–47. doi: 10.2527/jas.2015-9201
13. van Huis A. Potential of insects as food and feed in assuring food security. *Annu Rev Èntomol.* (2013) 58:563–83. doi: 10.1146/annurev-ento-120811-153704
14. Payne CLR, Dobermann D, Forkes A, House J, Josephs J, McBride A, et al. Insects as food and feed: European perspectives on recent research and future priorities. *J Insectsas Food Feed.* (2016) 2:269–76. doi: 10.3920/JIFF2016.0011
15. Finke MD, Rojo S, Roos N, van Huis A, Yen AL. The European Food Safety Authority scientific opinion on a risk profile related to production and consumption of insects as food and feed. *J Insects as Food Feed.* (2015) 1:245–7. doi: 10.3920/JIFF2015.x006
16. Acar U, Giannetto A, Giannetto D, Kesbiç OS, Yilmaz S, Romano A, et al. Evaluation of an innovative and sustainable pre-commercial compound as replacement of fish meal in diets for rainbow trout during pre-fattening phase: effects on growth performances, haematological parameters and fillet quality traits. *Animals.* (2021) 11:3547. doi: 10.3390/ani11123547
17. Boukid F, Riudavets J. del Arco L, Castellari M. Impact of diets including agro-industrial by-products on the fatty acid and sterol Profiles of larvae biomass from ephestiakuehniella, tenebrio molitor and hermetiaillucens. *Insects.* (2021) 12:672. doi: 10.3390/insects12080672
18. Kroeckel S, Harjes A-GE, Roth I, Katz H, Wuertz S, Susenbeth A, et al. When a turbot catches a fly: evaluation of a pre-pupae-meal of the Black Soldier Fly (*Hermetiaillucens*) as fishmeal substitute - Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*. (2012) 364–5:345–52. doi: 10.1016/j.aquaculture.2012.08.041
19. Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, De Smet S, et al. Black soldierflyasdietaryprotein source for broilerquails: apparentdigestibility, excretamicrobial load, feed choice, performance, carcass and meat traits. *Animal.* (2016) 10:1923–30. doi: 10.1017/S1751731116001270
20. Barroso FG, De Haro C, Sánchez-Muros M-J, Venegas E, Martínez-Sánchez A, Pérez-Bañón C. The potential of various insect species for use as food for fish. *Aquaculture*. (2014) 422–3:193–201. doi: 10.1016/j.aquaculture.2013.12.024
21. Yu GH, Chen YH, Yu ZN, Cheng P. Research progress on the larvae and prepupae of black soldier fly *Hermetiaillucens* used as animal feedstuff. *Chin Bull Entomol.* (2009) 46:41–5.
22. Bondari K. Sheppard DC. SoldierflyHermetiaillucens L, as feed for channelcatfish, Ictaluruspunctatus (Rafinesque), and blue tilapia, Oreochromisaureus (Steindachner). *Aquacult Fish Manage.* (1987) 18:209–20. doi: 10.1111/j.1365-2109.1987.tb00141.x
23. European Commission. Commission Regulation (EU) 2017/893 amending Annexes I and IV to Regulation (EC) No. 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. *Off J Eur Union.* (2017) 138:92–116.
24. Grigorakis K. Compositional and organoleptic quality of farmed and wild gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchuslabrax*) and factors affecting it: a review. *Aquaculture.* (2007) 272:55–75. doi: 10.1016/j.aquaculture.2007.04.062
25. Lock ER, Arsiwalla T, Waagbø R. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. *AquacultNutr.* (2015) 22:1202–13. doi: 10.1111/anu.12343
26. St-Hilaire S, Cranfill K, McGuire MA, Mosley EE, Tomberlin JK, Newton L, et al. Fish offal recycling by the black soldier fly produces a foodstuff high in Omega-3 fatty acids. *J World AquacSoc.* (2007) 38:309–13. doi: 10.1111/j.1749-7345.2007.00101.x
27. St-Hilaire S, Sheppard C, Tomberlin J, Irving S, Newton L, McGuire M, et al. Fly prepupae as a feedstuff for rainbow trout, oncorhynchus mykiss. *J World AquacSoc.* (2007) 38:59–67. doi: 10.1111/j.1749-7345.2006.00073.x
28. Ewald N, Vidakovic A, Langeland M, Kiessling A, Sampels S, Lalander C. Fatty acid composition of black soldier fly larvae (*Hermetiaillucens*) - possibilities and limitations for modification through diet. *Waste Manag.* (2020) 102:40–7. doi: 10.1016/j.wasman.2019.10.014
29. Barragan-Fonseca KB, Dicke M. Loon JJAV. Nutritional value of the black soldier fly (Hermetiaillucens L) and its suitability as animal feed - a review. *J Insects as Food Feed.* (2017) 3:105–20. doi: 10.3920/JIFF2016.0055
30. Meneguz M, Schiavone A, Gai F, Dama A, Lussiana C, Renna M, et al. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetiaillucens*) larvae. *J Sci Food Agric.* (2018) 98:5776–84. doi: 10.1002/jsfa.9127
31. Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, et al. Nutritionalcomposition of black soldierfly (*Hermetiaillucens*) prepupaereared on different organic wastesubstrates. *J Sci Food Agric.* (2017) 97:2594–600. doi: 10.1002/jsfa.8081
32. Liland NS, Biancarosa I, Araujo P, Biemans D, Bruckner CG, Waagbø R, et al. Modulation of nutrient composition of black soldier fly (*Hermetiaillucens*) larvae by feeding seaweed-enriched media. *PLoS ONE.* (2017) 12:e0183188. doi: 10.1371/journal.pone.0183188
33. Oteri M, Di Rosa AR, Lo Presti V, Giarratana F, Toscano G, Chiofalo B. Black Soldier Fly Larvae Meal as Alternative to Fish Meal for Aquaculture Feed. *Sustainability.* (2021) 13:5447. doi: 10.3390/su13105447
34. Di Rosa AR, Oteri M, Lo Presti V, Chiofalo B. Feed insects for aquaculture. Use of *Hermetiaillucens* L. meal for *Sparus aurata* L.: Emerging trends of advanced sensor-based instruments (E-eye, E-nose, E-tongue) for fish quality. In: Taylor, Francis, editor. *ASPA 24th Congress Book of Abstract*. Padua: Italy (2021). p. 49.
35. European Directive. Legislative Decree No. 26 implementing European Directive 2010/63/EU of the European Parliament and of the Council of 22

September 2010 on the protection of animals used for scientific purposes. *Off J Ital Leg.* (2014) 61:1–36.
36. European Directive. European Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J Eur.* (2010) 276:33–79.
37. Official methods of analysis of AOAC international. In: *21st Association of Official Analytical Chemists*. Gaithersburg, MD: Official methods of analysis of AOAC international (2019).
38. Folch J, Lees M, Stanley GHS A. Simple method for the isolation and purification of total lipides from animal tissues. *J BiolChem.* (1957) 226:497–509. doi: 10.1016/S0021-9258(18)64849-5
39. Christie WW. Preparation of ester derivatives of fatty acids for chromatographic analysis. In: Christie WW, editor. *Advances in Lipid Methodology-Two*. Dundee: Oily Press (1993). p. 69-111.
40. Ulbricht TL, Southgate DA. Coronary heart disease: seven dietary factors. *Lancet.* (1991) 338:985–92. doi: 10.1016/0140-6736(91)91846-M
41. Santos-Silva J, Bessa RJB, Santos-Silva F. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II Fatty acid composition of meat. *Livest Prod Sci.* (2002) 77:187–92. doi: 10.1016/S0301-6226(02)00059-3
42. Luciano G, Pauselli M, Servili M, Mourvaki E, Serra A, Monahan FJ, et al. Dietary olive cake reduces the oxidation of lipids, including cholesterol, in lamb meat enriched in polyunsaturated fatty acids. *Meat Sci.* (2013) 93:703–14. doi: 10.1016/j.meatsci.2012.11.033
43. Mustăţea G, Ungureanu EL, Iorga E. Protein acidic hydrolysis for amino acids analysis in food - progress over time: a short review. *J Hyg Eng Des.* (2019) 26:81–7.
44. Dogan G, Osman EO. Determination of amino acid and fatty acid composition of goldband goatfish [*Upeneusmoluccensis* (Bleeker, 1855)] fishing from the Gulf of Antalya (Turkey). *Int Aquat Res.* (2017) 9:313–27. doi: 10.1007/s40071-017-0179-9
45. ISO 21528-2:2017. *Microbiology of the Food Chain-Horizontal Method for the Detection and Enumeration of Enterobacteriaceae - Part 2: Colony-Count Technique*. Geneva: International Organization for Standardization (2017).
46. ISO 15213:2003. *Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Enumeration of Sulfite-Reducing Bacteria Growing under Anaerobic Conditions*. Geneva: International Organization for Standardization (2003).
47. ISO 6579-1:2017. *Microbiology of the Food Chain-Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella - Part 1: Detection of Salmonella spp*. Geneva: International Organization for Standardization (2017).
48. ISO 21872-1:2017. *Microbiology of the Food Chain-Horizontal Method for the determination of Vibrio spp. - Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus*. Geneva: International Organization for Standardization (2017).
49. Gram L, Trolle G, Huss HH. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. *Int J Food Microbiol.* (1987) 4:65–72. doi: 10.1016/0168-1605(87)90060-2
50. ISO 11290-1:2017. *Microbiology of the Food Chain-Horizontal Method for the Detection and Enumeration of Listeria Monocytogenes and of Listeria spp. - Part 1: Detection Method*. Geneva: International Organization for Standardization (2017).
51. Addinsoft. *XLSTAT Statistical and Data Analysis Solution*. New York, NY: Addinsoft (2022).
52. Food and Agriculture Organization. *Food and Agriculture Organization of the United Nations Minerals*. Rome: Food and Agriculture Organization (2016).
53. Iaconisi V, Marono S, Parisi G, Gasco L, Genovese L, Maricchiolo G, et al. Dietaryinclusion of Tenebrio molitor larvaemeal: effects on growth performance and finalqualitytreats of blackspotseabream (Pagellusbogaraveo). *Aquaculture.* (2017) 476:49–58. doi: 10.1016/j.aquaculture.2017.04.007
54. Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C. Sensoryanalysis of rainbowtrout, *Oncorhynchusmykiss*, fedenriched black soldierflyprepupae, *Hermetiaillucens*. *J World AquacultSoc.* (2011) 42:34–45. doi: 10.1111/j.1749-7345.2010.00441.x
55. Caballero MJ, Izquierdo MS, Kjørsvik E, Montero D, Socorro J, Fernández AJ, et al. Morphologicalaspects of intestinalcells from giltheadseabream (*Sparus aurata*) feddietscontainingdifferentlipid sources. *Aquaculture.* (2003) 225:325–40. doi: 10.1016/S0044-8486(03)00299-0
56. Pulido L, Secci G, Maricchiolo G, Gasco L, Gai F, Serra A, et al. Effect of dietary black soldier fly larvae meal on fatty acid composition of lipids and *sn*-2 position of triglycerides of marketable size gilthead sea bream fillets. *Aquaculture.* (2022) 546:737351–8. doi: 10.1016/j.aquaculture.2021.737351
57. HendersonRJ, Tocher DR. The lipid composition and biochemistry of freshwater fish. *Prog Lipid Res.* (1987) 26:281–347. doi: 10.1016/0163-7827(87)90002-6
58. Sargent JR, Tocher DR, Bell JG. The lipids. In: Halver J, Hardy E, editors. *Fish Nutrition.* 3rd ed. San Diego, CA: Academic (2002). p. 181–257.
59. SeiliezI, PanseratS, CorrazeG, Kaushik S, BergotP. Cloning and nutritional regulation of a Δ6-desaturase-like enzyme in the marine teleost gilthead seabream (Sparus aurata). *Comp Biochem Physiol B Biochem.* (2003) 135:449–60. doi: 10.1016/S1096-4959(03)00111-8
60. Tocher DR. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture.* (2015) 449:94–107. doi: 10.1016/j.aquaculture.2015.01.010
61. World Health Organization. Diet, Nutrition, and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation. Geneva: World Health Organization (2003).
62. Chen Y, Zhu X, Yang Y, Han D, Jin J, Xie S. Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance in gibel carp (*Carassius auratus gibelio*). *Aquacult Nutr.* (2014) 20:532–46. doi: 10.1111/anu.12106
63. Shiau SY Yu YP. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus×O. Aureaus Aquaculture.* (1999) 179:439–46. doi: 10.1016/S0044-8486(99)00177-5
64. Khoushab F, Yamabhai M. Chitin research revisited. *Mar Drugs.* (2010) 8:1988–2012. doi: 10.3390/md8071988
65. Palmegiano GB, Costanzo MT, Daprà F, Gai F, Galletta MG, Maricchiolo G, et al. Rice protein concentrate mealaspotentialdietaryingredient in practicaldiets for blackspotseabream (*Pagellusbogaraveo*). *AnimPhysiolAnimNutr.* (2007) 91:235–9. doi: 10.1111/j.1439-0396.2007.00697.x
66. Secci G, Parisi G. From farm to fork: lipid oxidation in fish products. *A review Ital J Anim Sci.* (2016) 15:124–36. doi: 10.1080/1828051X.2015.1128687
67. Weththasinghe P, Lagos L, Cortés M, Hansen JØ, Øverland M. Dietary Inclusion of Black Soldier Fly (*HermetiaIllucens*) Larvae Meal and Paste Improved Gut Health but Had Minor Effects on Skin Mucus Proteome and Immune Response in Atlantic Salmon (*Salmo Salar*). *Front Immunol.* (2021) 12:599530. doi: 10.3389/fimmu.2021.599530
68. Iaconisi V, Secci G, Sabatino G, Piccolo G, Gasco L, Papini AM. Effect of mealworm (Tenebrio molitor L) larvaemeal on amino acid composition of giltheadseabream (Sparus aurata L) and rainbowtrout (Oncorhynchusmykiss W) fillets. *Aquaculture.* (2019) 513:734403. doi: 10.1016/j.aquaculture.2019.734403
69. Sheppard DC, Newton GL, Thompson SA, Savage S. A value added manure management system using the black soldier fly. *Bioresour Technol.* (1994) 50:275–9. doi: 10.1016/0960-8524(94)90102-3
70. Rust MB. Nutritional physiology. In: Halver JE, Hardy RW, editors. *Fish Nutrition*. New York, NY: Academic Press (2002). p. 368–446.
71. Abro R, Sundell K, Sandblom E, Sundh H, Brännäs E, Kiessling A, et al. Evaluation of chitinolytic activities and membrane integrity in guttissues of Arcticcharr (*Salvelinusalpinus*) fedfishmeal and zygomycetebiomass. *Comp Biochem Physiol B BiochemMol Biol.* (2014) 175:1–8. doi: 10.1016/j.cbpb.2014.06.003
72. Krogdahl A, Hemre G, Mommsen T. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *AquacultNutr.* (2005) 11:103–22. doi: 10.1111/j.1365-2095.2004.00327.x
73. Lindsay GJH, Walton MJ, Adron JW, Fletcher TC, Cho CY, Cowey CB. The growth of rainbow trout (Salmo gairdneri) given diets containing chitin and its relationship to chitinolytic enzymes and chitin digestibility. *Aquaculture.* (1984) 37:315–34. doi: 10.1016/0044-8486(84)90297-7
74. Zhang Y, Zhou Z, Liu Y, Cao Y, He S, Huo F, et al. High-yield production of a chitinase from Aeromonas veronii B565 as a potential feed supplement for warm-water aquaculture. *ApplMicrobiolBiotechnol.* (2014) 98:1651–62. doi: 10.1007/s00253-013-5023-6

75. Oliva-Teles A, Enes P, Peres H. Replacing fishmeal and fish oil in industrial aquafeed. In: Davis A, editor. *Feed and Feeding Practices in Aquaculture*. Waltham, MA: Woodhead Publishing limited (2015). p. 203–33.
76. Magalhães R, Sánchez-López A, Leal RS, Martínez-Llorens S, Oliva-Teles A, Peres H. Black soldier fly (Hermetiaillucens) pre-pupae meal as a fish meal replacement in diets for European seabass (Dicentrarchuslabrax). *Aquaculture*. (2017) 476:79–85. doi: 10.1016/j.aquaculture.2017.04.021
77. National Research Council. Minerals. In: *Nutrient Requirements of Fish and Shrimp*. Washington, CO: The National Academies Press (2011). p. 163–85.
78. Wu G, Wu Z, Dai Z, Yang Y, Wang W, Liu C, et al. Dietary requirements of "nutritionally non-essential amino acids" by animals and humans. *Amino Acids*. (2013) 44:1107–13. doi: 10.1007/s00726-012-1444-2
79. Food and Agriculture Organization of the United Nations. *Aquaculture Feed and Fertilizer Resources Information System*. Rome: Food and Agriculture Organization (2017).
80. Rodehutscord M, Pfeffer E. Requirement for phosphorus in rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 200 g. *Water Sci Technol*. (1995) 31:137–41. doi: 10.2166/wst.1995.0370
81. Pimentel-Rodriguez AM, Oliva-Teles A. Phosphorus requirements of gilthead sea bream (*Sparus aurata* L.) juveniles. *Aquac Res*. (2001) 32:157–61. doi: 10.1046/j.1355-557x.2001.00013.x
82. Al-Yousuf MH, El-Shahawi MS, Al-Ghais SM. Trace metals in liver, skin and muscle of Lethrinuslentjan fish species in relation to body length and sex. *Sci Total Environ*. (2000) 256:87–94. doi: 10.1016/S0048-9697(99)00363-0
83. Borucka-Jastrzebska E, Kawczuga D, Rajkowska M, Protasowicki M. Levels of micro-elements (Cu, Zn, Fe) and macroelements (Mg, Ca) in freshwater fish. *J Elementol*. (2009) 14:437–47. doi: 10.5601/jelem.2009.14.3.02
84. Perkowska A, Protasowicki M. Cadmium and lead in fishes in selected elements of Swidwie Lake ecosystem. *Acta IchthyolPiscat*. (2000) 30:71–84. doi: 10.3750/AIP2000.30.2.07
85. Roméo M, Siau Y, Sidoumou Z, Gnassia-Barelli M. Heavy metal distribution in different fish species from the Mauritania coast. *Sci Total Environ*. (1999) 232:169–75. doi: 10.1016/S0048-9697(99)00099-6
86. Kumar V, Makkar HPS, Becker K. Dietary inclusion of detoxified Jatropha curcas kernel meal, effects on growth performance and metabolic efficiency in common carp, Cyprinus carpio (Linnaeus). *Fish Physiol Biochem*. (2010) 36:1159–70. doi: 10.1007/s10695-010-9394-7
87. Yi Y, Yang Z, Zhang S. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. *Environ Pollut*. (2011) 159:2575–85. doi: 10.1016/j.envpol.2011.06.011
88. Ip CCM Li XD, Zhang G, Wong CSC, Zhang WL. Heavy metal and Pb isotopic compositions of aquatic organisms in the Pearl River Estuary, South China. *EnvironPollut*. (2005) 138:494–504. doi: 10.1016/j.envpol.2005.04.016
89. Tuzen M, Soylak M. Determination of trace metals in canned fish marketed in Turkey. *Food Chem*. (2007) 101:1378–82. doi: 10.1016/j.foodchem.2006.03.044
90. Gulec AK, Yildrim NC, Danabas D, Yildirim N. Some Haematological and BiochemicalParameters in Common Carp (*Cyprinus carpio* L., 1758) in Munzur River, Tunceli, Turkey. *Asian J Chem*. (2011) 23:910–2. Available online at: https://hdl.handle.net/20.500.12406/428
91. Gu YG, Lin Q, Huang HH, Wang LG, Ning JJ, Du FY. Heavy metals in fish tissues/stomach contents in four marine wild commercially valuable fish species from the western continental shelf of South China Sea. *Marine Poll Bull*. (2017) 114:1125–9. doi: 10.1016/j.marpolbul.2016.10.040
92. Aslam S, Yousafzai AM. Chromium toxicity in fish: a review article. *J Entomol Zool Stud*. (2017) 5:1483–8.
93. Bakshi A, Panigrahi AK. A comprehensive review on chromium induced alterations in fresh water fishes. *Toxicol Rep*. (2018) 5:440–7. doi: 10.1016/j.toxrep.2018.03.007
94. European Commission. *Commission Regulation (EC) No. 629/2008 of 2 July 2008 Amending Regulation (EC) No. 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs*. Brussels: European Commission (2008). p. L173/6–9.
95. Lall SP. The minerals. In: Halver JE, Hardy RW, editors. *Fish Nutrition*. London: Academic Press (2002). p. 259–308.
96. FAO/WHO. *Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium*. Geneva: WHO Technical Report, Series No. 505 (1989).
97. Watanabe T, Kiron V, Satoh S. Trace minerals in fishnutrition. *Aquaculture*. (1997) 151:185–207. doi: 10.1016/S0044-8486(96)01503-7
98. Sivaperumal P, Sankar TV, Viswanathan Nair PG. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards. *Food Chem*. (2007) 102:612–20. doi: 10.1016/j.foodchem.2006.05.041
99. Ikem A, Egiebor NO. Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). *J Food Comp Anal*. (2005) 18:771–87. doi: 10.1016/j.jfca.2004.11.002
100. Forti E, Salovaara S, Cetin Y, Bulgheroni A, Tessadri R, Jennings P, et al. In vitro evaluation of the toxicity induced by nickel soluble and particulate forms in human airway epithelial cells. *Toxicol In Vitro*. (2011) 25:454–61. doi: 10.1016/j.tiv.2010.11.013
101. Oliva-Teles A. Nutrition and health of aquaculture fish. *J Fish Dis*. (2012) 35:83–108. doi: 10.1111/j.1365-2761.2011.01333.x
102. Tang Q, Wang C, Xie C, Jin J, Huang Y. Dietary available phosphorus affected growth performance, body composition, and hepatic antioxidant property of juvenile yellow catfish (*Pelteobagrusfulvidraco*). *Sci World J*. (2012) 2012:987570. doi: 10.1100/2012/987570
103. de Bruijn I, Liu Y, Wiegertjes GF, Raaijmakers JM. Exploring fish microbial communities to mitigate emerging diseases in aquaculture. *FEMS Microbiol Ecol*. (2018) 94:fix161. doi: 10.1093/femsec/fix161
104. Rurangwa E, Verdegem MC. Microorganisms in recirculating aquaculture systems and their management. *RevAquac*. (2015) 7:117–30. doi: 10.1111/raq.12057

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Contribution of Tocopherols in Commonly Consumed Foods to Estimated Tocopherol Intake in the Chinese Diet

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Vitamin E is an essential fat-soluble nutrient mainly found in vegetable oils, nuts, and other foods. In this study, we evaluated the contribution of commonly consumed foods to the vitamin E dietary intake of the population in relation to their consumption practices. In addition, the vitamin E intakes of Chinese residents were compared in different regions of China and in different years. The results showed that vegetable oil was the main source of vitamin E dietary intake for Chinese residents, accounting for 46.76% of total dietary intake of vitamin E, followed by cereals, vegetables, meat, aquatic products, eggs, legumes, nuts, fruits and dairy products. Among all vegetable oils, rapeseed oil was the highest contributor of vitamin E, accounting for 10.73% of all foods. Due to dietary habits and regional differences, vitamin E intake also varies greatly among residents in different regions of China and has increased yearly from 1982 to 2020. This study provides with scientific evidence for reasonable VE supplementation.

Keywords: China, vitamin E, vegetable oil, dietary intake, dietary supplementation

INTRODUCTION

Vitamin E (VE) is an essential fat-soluble nutrient in numerous foods such as nuts, seeds and vegetable oils. As a natural antioxidant, VE could stop the production of reactive oxygen species (ROS) formed when fat undergoes oxidation. In addition, VE is of clinical importance for the modulation of immune function, as it affects the susceptibility of the host to infectious diseases (1). Moreover, VE was found to improve vascular function in diabetic patients (2). Together with vitamin C, VE significantly improved cognitive function in old mice (3). The previous showed that a moderate VE supplementation significantly protected sperm quality in males and egg quality in females by reducing lipid peroxidation in sperm (4). Dietary supplementation with 50–100 mg/kg vitamin E significantly improved the growth and intestinal performance of animals, increasing the activity of several digestive enzymes. Furthermore, VE supplementation significantly increased the height of the intestinal folds and the thickness of the mucosa (5). Theoretically, the functions of different tocopherols vary with their structure (6). For example, γ-tocopherol was effective in inhibiting colon and lung cancer, and controlling cancer progression, while δ-tocopherol was superior to α-tocopherol and γ-tocopherol in tumor-inhibiting activity (7). Moreover, α-tocopherol is also found to be a reflection of daily dietary status (8). All tocopherols except β-tocopherol inhibit smooth muscle proliferation, and α-tocopherol is also not the only isomer important for human health (9). More importantly, no adverse effect from consuming VE in food was reported. Only VE supplements at very high dose (>1,000 mg/day) might lead pro-oxidant damage (10).

Both plant-based diets and animal products are important sources of VE, with the amount varying in different foods. Nuts, seeds, and vegetable oils contain high levels of tocopherols, as do green leafy vegetables and fortified cereals (11). The dietary intake of VE varies from country to country, and also from time to time, due to different dietary structures and eating habits. To increase VE intake, many VE fortified products such as VE encapsulation appeared in market (12). However, it is not clear whether the body is deficient in vitamin E or not. Without dietary nutritional data, overconsumption of VE fortified products often occurs. Therefore, it is necessary to assess the dietary intake of the population to determine whether different groups of people lack VE while also providing scientific and reasonable guidance on VE supplementation.

Dietary assessment is an important tool for the scientific guidance of a diet. Dietary assessment mainly focuses on the comprehensive assessment of the dietary intake of individuals and provides assessment results. Among existing dietary assessment methods, food frequency questionnaires (FFQs) have been widely used in large epidemiological studies since the 1990s (13). After its accuracy was questioned in the early 2000s, many changes in assessment methods occurred. Some researchers shifted their attentions to improving the feasibility and accuracy of open-ended dietary assessment methods rather than improving the FFQ or further searching for relevant biomarkers, while other researchers focused on improving the accuracy of the FFQ (14). The dietary consumption and nutritional status of women undergoing chemotherapy were evaluated for breast cancer (15). In our previous study, the content and composition of phytosterols in different vegetable oils were analyzed to estimate the total intake of phytosterols and the contribution of food to nutrient intake based on consumption data (16). Three national surveys in the United States including the 2001–2002 National Health and Nutrition Examination Survey (NHANES), NHANES III (1988–1994), and the Continuing Survey of Food Intakes by Individuals (1994–1996) were conducted to assess the intake levels of vitamin E of the diets of most Americans (17). At present, the composition and determination of vitamin E among the important evaluation criteria of vegetable oils are less studied. In addition, there are fewer studies on the composition and determination of vitamin E. Therefore, the intake of vitamin E for the population is still unclear. Moreover, there is no systematic study to assess the intake of vitamin E in Chinese populations. So, it is necessary to conduct this dietary assessment.

In this study, the composition and content of vitamin E in main kinds of foods in Chinese daily diets were summarized, and the amount of VE intake was calculated. Moreover, the distribution in different regions and historical changes in the VE intake of Chinese residents were analyzed for reasonable diet and appropriate nutritional supplements.

MATERIALS AND METHODS

Data Source

Data on the tocopherol content and composition in cereals, grains, potatoes, legumes, vegetables, fruits, nuts, vegetable oils, meat, eggs, milk, and aquatic products used in this article were obtained from the Chinese Food Composition Tables released by National Institute for Nutrition and Health, Chinese Center for Disease Control and Presentation. In this database, there are more than 3.3 thousand kinds of plant derived foods and more than 3.6 thousand kinds of animal derived foods. China's domestic consumptions of main kinds of foods were obtained from production, supply, and distribution (PSD) reports released by United States Department of Agriculture (USDA), China Statistical Yearbook, and China Population Nutrition and Health Status Monitoring Report. From the China Population Nutrition and Health Status Monitoring Report and USDA PSD reports, the consumption of major foods of Chinese residents, including cereals, potatoes, beans, vegetables, fruits, nuts, vegetable oil, meat, eggs, milk, and aquatic products. As shown in **Supplementary Table 1**, beans include soybeans, mung beans, and red beans, which account for the largest consumption proportion in China, while 26 vegetables are selected that Chinese residents consume the most and frequently. The source of fruit consumption in USDA PSD reports mainly includes apple, banana, pear, grape, peach, orange, orange, grapefruit, and cherry. Finally, taking the tocopherol content and consumption into consideration, as shown in **Supplementary Tables 1**, **2**, 60 kinds of foods and 12 kinds of vegetable oils were selected to assess the estimated tocopherol intake in the Chinese diet.

Calculation Method

Although the four chemical structures of tocopherols are similar, their biological activities are different. The body absorbs different tocopherols to different degrees. α-Tocopherol is the most widely distributed, while the activity of β-, γ-, and δ-tocopherol is 50, 10, and 2% of that of α-tocopherol, respectively. Therefore, the amount of VE in food is often expressed as α-tocopherol equivalent (α-TE) and could be calculated by the following conversion formula.

$$\begin{aligned}\alpha\text{-TE (mg)} = &\ \alpha\text{-tocopherol (mg)} + 0.5 \times \beta\text{-tocopherol (mg)} \\ &+ 0.1 \times \gamma\text{-tocopherol (mg)} + 0.01 \\ &\times \delta\text{-tocopherol (mg)}\end{aligned} \tag{1}$$

Firstly, α-TE values of commonly consumed foods were calculated by the levels of tocopherols in various foods recorded in Chinese Food Composition Tables and the above formula, and shown in **Supplementary Tables 1**, **2**. Then, the estimated VE intake from each food was calculated by product of α-TE values and the daily consumption of commonly consumed foods queried in the USDA and China Statistical Yearbook, respectively. Finally, the estimated tocopherol intake in the Chinese diet was calculated by sum of the estimated VE intakes from commonly consumed foods. Moreover, we also calculated the contribution of a certain kind of foods to total VE intake (16).

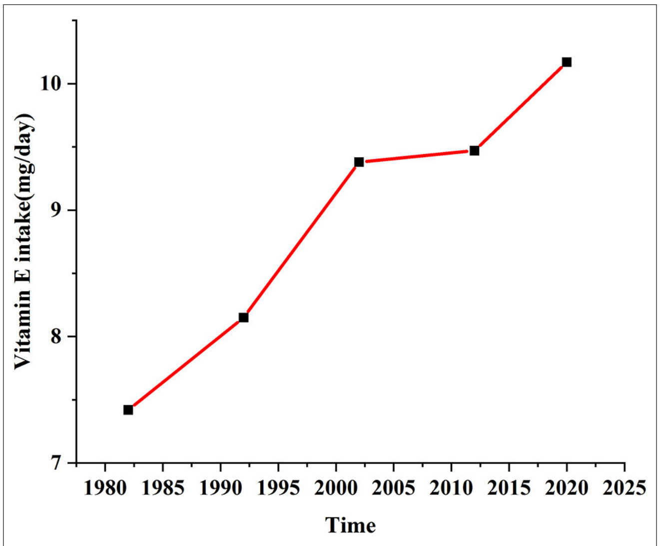

FIGURE 1 | Changes in vitamin E intake of Chinese residents between 1982 and 2020.

Moreover, although α-TE is commonly used to assess the VE content in foods, other isomers also have different functions and effects on an organism. For example, as abovementioned, δ-tocopherol was superior to α-tocopherol in tumor-inhibiting activity (7). Thus, we also assessed the estimated VE intake calculated by product of the total amount of tocopherols and the daily consumption of commonly consumed foods queried in the USDA and China Statistical Yearbook.

The data in the schematic diagram of VE intake of Chinese residents in the past 40 years mentioned in **Figure 1** are from the Chinese food composition table, the monitoring report on the nutrition and health status of Chinese residents, the outline of food and nutrition development in China, the 1992 national nutrition survey, and the 2001–2012 monitoring of nutrition and health of Chinese residents. Based on the consumption of vegetable oil in 1982, 1992, 2002, 2012, and 2020, and then combined with the contents of VE in vegetable oils, the VE intake of Chinese residents from vegetable oils in each year was calculated.

RESULTS

Contents and Compositions of Tocopherols in Different Foods

VE is synthesized only in photosynthetic organisms, including higher plants. A balanced dietary pattern for Chinese residents covers several major categories of essential basic foods, including cereal, vegetable, fruit, livestock, poultry, fish, egg, milk, bean, nut, and cooking oils. Since the contents of tocotrienols in foods are usually low compared with those of tocopherols and do not affect the assessment of VE dietary intake (18). Therefore, we selected the tocopherols for the calculation. In this study, the contents of tocopherols in 12 kinds of major foods including cereal, grain, potato, bean, vegetable, fruit, nut, vegetable oil, meat, egg, milk, and aquatic products were collected and shown in **Supplementary Tables 1**, **2**. The highest content of tocopherols was found in vegetable oils, such as sunflower oil. Vegetable oils mainly contain α-tocopherol and γ-tocopherol, as their main source of antioxidants (19). This is followed by nuts, with a weighted average tocopherol content of 165.53 mg kg^{-1}. Some studies have shown that the oils extracted from walnuts, almonds, peanuts, hazelnuts, and macadamia nuts are a good source of tocopherols (20, 21). Meanwhile, cereal grains, eggs, and aquatic products also contain some amount of tocopherols. The tocopherol content of different types of grains varies greatly. As shown in **Supplementary Table 1**, wheat has the higher VE content than rice. Although the amount of tocopherols in fish varies with different species, α-tocopherol is the predominant form of VE (22).

VE was first discovered in green leafy vegetables in 1922, and it was found to be an essential nutrient for plant reproduction (23). However, vegetables, fruits, and dairy products were found to have lower levels of tocopherols compared to vegetable oils and nuts. Cruciferous vegetables are a good source of antioxidants, and the antioxidant content varies considerably both within and between subspecies (24, 25).

Palm, soybean, rapeseed, sunflower seed, corn, and coconut oil are the most exploited vegetable oils worldwide, which account for 93% consumption of vegetable oils (26). In China, peanut oil, sesame oil, flaxseed oil, Camellia oil, cottonseed oil, olive oil, and grape seed oil are also important source to intake VE. So, as shown in **Supplementary Table 2**, composition and content of tocopherols in 12 kinds of vegetable oils were collected and analyzed in this study. The tocopherols in vegetable oils are mainly α-, γ-, and δ-tocopherols. The content and composition of tocopherols in vegetable oils are presented in **Supplementary Table 2**. Soybean oil had the highest total tocopherol content of 930.80 mg kg^{-1}, high γ-tocopherol and δ-tocopherol content, and very low α-tocopherol content. Palm oil is very low in tocopherols, with the total tocopherol content of 152.40 mg kg^{-1}, and is mainly produced in Malaysia and Indonesia. The total content of tocopherols in rapeseed oil is 608.90 mg kg^{-1}, and the main components are α-tocopherol and γ-tocopherol. The types of tocopherols in corn oil and rapeseed oil are similar, mainly containing α- and γ-tocopherols, with a total tocopherol content of 509.40 mg kg^{-1}. Sunflower oil ranks fourth in consumption after soybean oil, rapeseed oil, and palm oil, with a total tocopherol content of 546.00 mg kg^{-1}. The total tocopherol content of peanut oil was 420.60 mg kg^{-1} and mainly contained α and γ species. Olive oil is known in the West as "liquid gold." The total tocopherol content in olive oil is 169.10 mg/kg, with the high content of α-tocopherol, accounting for 85% of the total tocopherol content. Moreover, sesame oil is a traditional Chinese flavored vegetable oil, and the total content of tocopherols in sesame oil is 685.30 mg kg^{-1} (mainly consisting of γ-tocopherol). Flaxseed oil and cottonseed oil include mainly γ-tocopherol (27).

Assessment of Dietary Intake of VE in the Chinese Population

Combining the tocopherol levels of vegetable oils and other foods, we reviewed the tocopherol levels of a total of 10 food items, excluding vegetable oils, covering almost all food groups in the daily diet of the Chinese population, including cereals,

TABLE 1 | Contribution of commonly consumed foods to vitamin E dietary intake.

Food categories	Consumption (g/day)	α-TE (mg/kg)	Total tocopherol content (mg/kg)	Intake of vitamin E (mg/day)	Percent of total intake of vitamin E (%)
Cereal	377.00	4.92	9.90	1.85	18.45
Coarse cereals	28.00	7.49	21.73	0.21	2.09
Potato	36.00	1.86	3.10	0.07	0.67
Bean	11.00	21.29	183.49	0.23	2.33
Vegetable	269.00	3.56	5.55	0.96	9.53
Fruit	41.00	4.66	8.20	0.19	1.90
Nut	4.00	50.08	165.53	0.20	1.99
Vegetable oil	37.00	128.55	642.01	4.76	46.76
Meat	104.00	6.15	8.71	0.64	6.36
Egg	33.00	12.68	19.48	0.42	4.16
Milk	66.00	1.38	1.70	0.09	0.90
Aquatic products	45.00	12.25	22.35	0.55	5.48
Total				10.17	100.00

Data on consumption of various types of foods are from the United States Department of Agriculture (USDA), the 2020 China Statistical Yearbook, and the China Population Nutrition and Health Status Monitoring Report.

TABLE 2 | Contribution of vegetable oils to vitamin E dietary intake.

Vegetable oil type	Tocopherol content (mg/kg)	Consumption ratio (%)	Vitamin E intake (mg/day)	Contribution of vitamin E (%)
Soybean oil	61.10	43.00	0.97	9.56
Rapeseed oil	147.50	20.00	1.09	10.73
Palm oil	128.82	17.00	0.81	7.97
Peanut oil	194.34	8.00	0.58	5.66
Sunflower oil	450.83	6.00	1.00	9.84
Other oils	138.03	6.00	0.31	3.01

potatoes, legumes, vegetables, fruits, nuts, meat, eggs, dairy products, and aquatic products. For food with low consumption frequency and consumption, their impact on the assessment of the overall VE intake of the Chinese population was minimal. α-Tocopherol has a very important antioxidant effect on cell membranes (28) and is also more effective in improving oxidative stability and reducing the relative oxidation rate with increasing temperature (29). In this study, we used the total content of tocopherols and the α-TE to assessment of dietary intake of VE in the Chinese population.

The contributions of different foods and vegetable oils to the VE intake of the Chinese population are listed in **Tables 1**, **2**, respectively. The total VE intake (calculated by α-TE) from the daily diet of the residents was 10.17 mg day^{-1}, while the recommended dietary reference intake (AI) of VE for adults in China is 14.0 mg day^{-1}. Comparing the results, the actual VE intake of Chinese residents did not meet the recommended intake. The contribution of vegetable oil to VE intake ranked first in the dietary intake of residents, accounting for 46.76% of VE intake (4.76 mg day^{-1}), while cereals ranked second with 18.24% (1.85 mg day^{-1}), followed by vegetables at 9.42% (0.96 mg day^{-1}). The highest contributing vegetable oil was rapeseed oil, accounting for 10.73% (1.09 mg day^{-1}) of the total VE intake. Although soybean oil had the highest total tocopherol content, its lower α-tocopherol content meant that its contribution toward dietary intake was lower than that of rapeseed oil.

Though traditional VE dietary intake assessment was conducted by using α-tocopherol equivalents, other tocopherols show various functions different from α-Tocopherol. For example, γ-Tocopherol is found to be a more potent free radical scavenger than α-tocopherol *in vitro* (30). Therefore, we performed VE dietary intake assessment by using the total content of tocopherols. The results were shown in **Supplementary Table 3**. The VE intake of Chinese residents was 35.38 mg day^{-1} per capita. Among the 12 kinds of foods, vegetable oils contributed the most (>50%), followed by cereals. These results were almost consistent with those of the α-TE.

DISCUSSION

Estimated Dietary VE Intake of Residents in Different Dietary Pattern of China

China is a large geographical area, and residents of different regions have different dietary habits. The different kinds of cereals and edible oils are the main differences of dietary habits for residents of different regions in China. Therefore, we selected several regions in China with different dietary structures to analyze and compare VE dietary intake. In this section, the estimated VE intake from cereals or edible oils was calculated by product of α-TE value of a particular kind of edible oil (like rapeseed oil) or cereal (like wheat) and the daily consumption of edible oils or cereals queried in the USDA and China Statistical Yearbook. The results are presented in **Table 3**.

The distribution of edible oil varieties in China shows obvious geographical differences. The northwestern region such as Gansu province is vast in area but also arid and water-scarce, with fragile ecological conditions. The Qinghai and Tibetan regions are the main settlements of Tibetans, and the region has very little

TABLE 3 | Estimated vitamin E intake of residents in different dietary pattern of China.

Dietary pattern	Classic region	Vitamin E intake (mg/day)
Soybean oil+ Wheat	Inner Mongolia, Hebei, Shanxi	7.81
Soybean oil+ Rice	Heilongjiang, Jilin, Liaoning	6.79
Rapeseed oil+ Wheat	Qinghai	11.04
Rapeseed oil+ Rice	Hubei, Hunan, Shanghai, Zhejiang, Jiangsu, Anhui, Jiangxi, Sichuan	10.02
Peanut oil+ Wheat	Henan, Shandong, Anhui	12.77
Peanut oil+ Rice	Guangdong, Guangxi, Hong Kong, Macao	11.75
Camellia oil+ Rice	Camellia Oil Belt from Yunnan to Zhejiang	5.54
Sunflower seed Oil+ Wheat	Xinjiang	22.26
Soybean oil+ Indica rice	Taiwan	7.09
Butter +Highland barely	Tibet	6.80

land suitable for cultivation, with its alpine climate. The main crop in the region is barley. So, the daily diet in this region has obvious regional characteristics. The vitamin sources available to the residents in this region mainly come from barley and ghee. The VE intake of the residents in this region (6.80 mg day^{-1}) is lower than the national average, indicating serious VE deficiency.

The northeastern China's Heilongjiang, Jilin, and Liaoning province have a dietary structure consisting of soybean oil and rice. Thus, VE supplementation is essential for residents of these areas. Meanwhile, the tocopherols were obtained primarily from soybean oil and wheat and also showed insufficient VE intake (7.81 mg day^{-1}) for residents in Hebei, Shanxi, Shaanxi and Inner Mongolia. Residents in Henan, Shandong, Anhui, Guangxi, Guangdong, Hong Kong, and Macao had relatively high consumption rates of peanut oil, while those in South China had a sufficient intake of fresh vegetables and aquatic products rich in VE. Residents in areas in the Yangtze River basin such as Hubei and Hunan mainly consumed rapeseed oil, and the VE content of both peanut oil and rapeseed oil is relatively high. Edible oils in Shanghai, Zhejiang, Jiangsu, Anhui, and Jiangxi are also dominated by rapeseed oil, and the intake of VE of residents in these areas is 10.02 mg day^{-1}, which is close to the national average. However, since the VE content of tea oil is lower, while the tocopherol content of rice is also lower compared to wheat and other grains, residents from these regions had the lowest national VE intake of 5.54 mg day^{-1}. The geographical conditions in the south are complex, and there are differences in dietary habits and dietary structure in different provinces. This causes the VE intake to vary among residents of different regions in the south. The dietary structure of China has long been influenced by the living environment and is adapted to local conditions. So, the dietary intake structure of residents in different regions varies and results in very different levels of VE in the dietary intake.

In summary, Chinese residents do not consume enough VE. There is no doubt that supplementing VE through food is the healthiest option. Edible oil is an essential part of our daily diet, which not only improves the color of dishes and adds flavor to food but also provides a rich source of VE. Among main edible oils, rapeseed oil, peanut oil, and sunflower oil have high tocopherol content and are a good choice for VE supplementation. Meanwhile, it is necessary to develop some high VE edible oils including *Eucommia ulmoides* Oliver seed oil and sea buckthorn seed oil (31) to improve the dietary intake of VE, especially for residents with low VE intake.

Historical Dietary Intake of VE by Chinese Residents

Over the past 40 years, the dietary structure of Chinese residents has changed dramatically. In the past, residents received most of their calories from grains and fats, but today, the intake of fruits and vegetables has gradually increased. VE intake from 1982 to 2020 in China are illustrated in **Figure 1**. The consumption of vegetable oil has gradually increased, and the intake of VE by Chinese residents has also gradually increased. VE intake among Chinese residents has increased year by year since 1982, showing an upward trend. Economic and social development has led to a dramatic improvement in the standard of living of Chinese residents. In just four decades, China has solved the problem of feeding 1.3 billion people and is now dealing with an increasing number of nationals who are overweight, obese, or dealing with nutritional imbalance due to food-borne diseases.

The structure of the food consumption of residents has undergone different changes in various periods. As the income level of the population increased and due to the advancement of industrialization and urbanization, there were two more significant changes in the food consumption of the populace. First, the per capita consumption of cereal products continued to rise until 1984 but exhibited a downward trend thereafter. Second, the consumption of animal products, such as livestock and poultry meat, aquatic products, and dairy products, as well as fruits and vegetables, increased significantly. Although the consumption of fruits and vegetables has increased, it still has not reached the 500 g day^{-1} level recommended in the Dietary Guidelines for Chinese Residents. In recent years, the domestic demand for vegetable oils has been on the rise. Therefore, selecting and breeding high VE oil crops or improving the VE content in edible oil and oilseed products by other means are very important to improve the national dietary health level and achieving improved national physical fitness through the adjustment of dietary structure.

CONCLUSION

Overall, the current daily dietary intake of VE by Chinese residents does not meet the needs of the human body. From the calculations in the previous sections, it was concluded that vegetable oils are the largest contributor of VE in a daily diet; so, the choice and quality of vegetable oil varieties affect the VE intake of Chinese residents. Among several bulk vegetable oils consumed daily, soybean oil had the highest total tocopherol content. However, its α-tocopherol content was low, while canola and peanut oils had higher α-TE content and can be used as the main dietary source of VE supplementation. The VE intake of residents in different regions of China was further compared, and significant differences in the intake of VE in different regions were found due to differences in geography and dietary habits. The intake of VE by Chinese residents from 1982 to 2020 was also compared. Over the past 40 years, the dietary structure of Chinese residents has changed dramatically. Their food consumption has become diversified, and the purpose of food intake has changed from satisfying sustenance to maintaining health. The consumption of cereals has declined, and the consumption of fruits and vegetables has risen. Although the dietary nutrition of Chinese residents has improved, there is still much need to intake VE from foods. This study provides with scientific evidence for reasonable VE supplementation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YZ and LZ: methodology and writing—original draft. LZ: conceptualization and writing—review and editing. XQ, XueyW, XuefW, FM, LY, JM, and JJ: formal analysis. PL and LZ: funding acquisition. PL: supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.829091/full#supplementary-material

Supplementary Table 1 | Tocopherol contents of main foods except vegetable oils.

Supplementary Table 2 | Composition and content of tocopherols in main kinds of vegetable oils.

Supplementary Table 3 | The contribution of total tocopherol from main kinds of foods.

REFERENCES

1. Traber MG. Vitamin E. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins R, editors. *Modern Nutrition in Health and Disease*. 10th edition. Baltimore, MD: Lippincott Williams & Wilkins (2006). p. 396–411.
2. Alshiek JA, Dayan L, Asleh R, Blum S, Levy AP, Jacob G. Anti-oxidative treatment with vitamin E improves peripheral vascular function in patients with diabetes mellitus and haptoglobin 2-2 genotype: a double-blinded cross-over study. *Diabetes Res Clin Pract.* (2017) 131:200–7. doi: 10.1016/j.diabres.2017.06.026
3. Arzi A, Hemmati AA, Razian A. Effect of vitamins C and E on cognitive function in mouse. *Pharmacol Res.* (2004) 49:249–52. doi: 10.1016/j.phrs.2003.10.004
4. Rengaraj D, Hong YH. Effects of dietary vitamin E on fertility functions in poultry species. *Int J Mol Sci.* (2015) 16:9910–21. doi: 10.3390/ijms16059910
5. He M, Wang K, Liang X, Fang J, Geng Y, Chen Z, et al. Effects of dietary vitamin E on growth performance as well as intestinal structure and function of channel catfish (Ictalurus punctatus, Rafinesque 1818). *Exp Ther Med.* (2017) 14:5703–10. doi: 10.3892/etm.2017.5295
6. Eitenmiller R, Lee J. *Vitamin E: Food Chemistry, Composition and Analysis*. New York, NY: ImprintCRC Press (2004).
7. Li GX, Lee MJ, Liu AB, Yang Z, Lin Y, Shih WJ, et al. delta-tocopherol is more active than alpha - or gamma -tocopherol in inhibiting lung tumorigenesis in vivo. *Cancer Prev Res.* (2011) 4:404–13. doi: 10.1158/1940-6207.CAPR-10-0130
8. Bates CJ, Mishra GD, Prentice A. Gamma-tocopherol as a possible marker for nutrition-related risk: results from four national diet and nutrition surveys in Britain. *Br J Nutr.* (2004) 92:137–50. doi: 10.1079/BJN20041156
9. Saldeen K, Saldeen T. Importance of tocopherols beyond α-tocopherol: evidence from animal and human studies. *Nutr Res.* (2005) 25:877–89. doi: 10.1016/j.nutres.2005.09.019
10. Brown KM, Morrice PC, Duthie GG. Erythrocyte vitamin E and plasma ascorbate concentrations in relation to erythrocyte peroxidation in smokers and non-smokers: dose response of vitamin E supplementation. *Am J Clin Nutr.* (1997) 65:496–502. doi: 10.1093/ajcn/65.2.496
11. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S., Mahdi. The role of vitamin E in human health some diseases. *Sultan Qaboos Univ Med J.* (2014) 14:e157–e165.
12. Ribeiro AM, Estevinho BN, Rocha F. The progress and application of vitamin E encapsulation – a review. *Food Hydrocoll.* (2021) 121:106998. doi: 10.1016/j.foodhyd.2021.106998
13. Yao ZX, Dai K, Meng G, Zhang Q, Liu L, Wu HM, et al. Low dietary quercetin intake by food frequency questionnaire analysis is not associated with hypertension occurrence. *Clin Nutr.* (2021) 40:3748–53. doi: 10.1016/j.clnu.2021.04.047
14. Shim JS, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. *Epidemiol Health.* (2014) 36:e2014009. doi: 10.4178/epih/e2014009
15. Ro HK. Evaluation of vitamin E adequacy of a group of rural (Amish) people in the U.S.A. *J. Korean Soc Food Sci Nutr.* (1990) 19:207–14.
16. Yang RN, Xue L, Zhang LX, Wang X, Qi X, Jiang J, et al. Phytosterol contents of edible oils and their contributions to estimated phytosterol intake in the Chinese diet. *Foods.* (2019) 8:334. doi: 10.3390/foods8080334
17. Gao X, Wilde PE, Lichtenstein AH, Bermudez OI, Tucker KL. The maximal amount of dietary á-tocopherol intake in U.S. adults (NHANES 2001-2002). *J Nutr.* (2006) 136:1021–6. doi: 10.1093/jn/136.4.1021
18. Cerretani L, Lerma-Garcia MJ, Herrero-Martinez JM, Gallina-Toschi T, Simo-Alfonso EF. Determination of tocopherols and tocotrienols in vegetable oils by nanoliquid chromatography with ultraviolet-visible detection using a silica monolithic column. *J Agric Food Chem.* (2010) 58:757–61. doi: 10.1021/jf9031537
19. Ergönül PG, Köseoglu O. Changes in α-, β-, γ- and δ-tocopherol contents of mostly consumed vegetable oils during refining process. *CYTA J Food.* (2013) 12:199–202. doi: 10.1080/19476337.2013.821672
20. Maguire LS, O'Sullivan SM, Galvin K, O'Connor TP, O'Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *INT J Food Sci Nutr.* (2004) 55:171–8. doi: 10.1080/09637480410001725175
21. Imsanguan P, Roaysubtawee A, Borirak R, Pongamphai S, Douglas S, Douglas PL. Extraction of α-tocopherol and γ-oryzanol from rice bran. *ACS Food Sci Technol.* (2008) 41:1417–24. doi: 10.1016/j.lwt.2007.08.028

22. Polat A, ÖZogul Y, Kuley E, ÖZogul F, ÖZyurt G, SImSEk A. Tocopherol content of commercial fish species as affected by microwave cooking. *J Food Biochem.* (2013) 37:381–7. doi: 10.1111/j.1745-4514.2011.00635.x
23. Evans HM, Bishop KS. On the existence of a hiitherto unrecognized dietary factor essential for reproduction. *Science.* (1922) 56:650–1. doi: 10.1126/science.56.1458.650
24. Singh J, Upadhyay AK, Prasad K, Bahadur A, Rai M. Variability of carotenes, vitamin C, E and phenolics in brassica vegetables. *J Food Compost Anal.* (2007) 20:106–12. doi: 10.1016/j.jfca.2006.08.002
25. Williamson G. Protective effects of fruits and vegetables in the diet. *Food Sci Nutr.* (1996) 96:6–10. doi: 10.1108/00346659610105806
26. Mitrea L, Teleky BE, Leopold LF, Nemes SA, Plamada D, Dulf FV, et al. The physicochemical properties of five vegetable oils exposed at high temperature for a short-time-interval. *J Food Compos Anal.* (2022) 106:104305. doi: 10.1016/j.jfca.2021.104305
27. Ahmad S, Anwar F, Hussain AI, Ashraf M, Awan AR. Does soil salinity affect yield and composition of cottonseed oil? *J Am Oil Chem Soc.* (2007) 84:845–51. doi: 10.1007/s11746-007-1115-8
28. Gordon MH, Kourimska L. Effect of antioxidants on losses of tocopherols. *Food Chem.* (1995) 52:175–7. doi: 10.1016/0308-8146(94)P4200-Y
29. Wong JW, Hashimoto K, Shibamoto T. Antioxidant activities of rosemary and sage extracts and vitamin-e in a model meat system. *J Agric Food Chem.* (1995) 43:2707–12. doi: 10.1021/jf00058a029
30. Duthie GG, Gonzalez BM, Morrice PC, Arthur JR. Inhibitory effects of isomers of tocopherol on lipid peroxidation of microsomes from vitamin E-deficient rats. *Free Radic Res Commun.* (1991) 15:35–40. doi: 10.3109/10715769109049123
31. Yang RN, Zhang LX, Li PW, Yu L, Mao J, Wang XP, et al. A review of chemical composition and nutritional properties of minor vegetable oils in China. *Trends Food Sci Technol.* (2018) 74:26–32. doi: 10.1016/j.tifs.2018.01.013

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Rural Market Food Diversity and Farm Production Diversity: Do They Complement or Substitute Each Other in Contributing to a Farm Household's Dietary Diversity?

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Majority of undernourished people live in rural Asia and Africa, and many of them are smallholder farmers and consume a significant amount of what they produce. This is specifically true in India. However, in the context of increasing commercial production systems, it is not well-known how much food is consumed from a particular food group that was purchased, what proportion of food is from the production of farm households, and how their diets change seasonally. Furthermore, whether the rural market food diversity complements or substitutes farm production diversity in household's diets is unknown. We employed a mixed-methods research design to answer these questions. The research was conducted in three villages in Telengana State. The results reveal that crop diversity has significantly declined from a highly-diverse production system to a less diverse one. The Food Consumption Score results show that on average own-farm production contributes 23% of food (mainly starchy staples), while market purchases contribute 77% of calories consumed (from more diverse and nutritious foods). Therefore, in the study, villages' market food diversity is more important, and it is complementary to own-farm production. However, our study shows that mere market access (the most widely used proxy indicator in the literature) does not guarantee the availability of diverse nutritious foods to households who use that specific market. This is because market food diversity varies from market to market and across seasons. Therefore, we proposed that in commercial production systems improving crop diversity, and strengthening rural markets, are needed. Moreover, incentivizing retail business and subsidizing nutritious and/or biofortified food in rural areas must be part of strategies to improve nutrition in rural India.

Keywords: market food diversity, dietary diversity, India, seasonality, food systems

INTRODUCTION

The rural poor are the most vulnerable, food insecure, and malnourished people in the world, and a significant population of them lives in rural Asia and Africa, and many of them are smallholder farm households that largely depend on agriculture for their livelihoods (Pinstrup-Andersen, 2007; Muller, 2009; Qaim et al., 2016; Gupta et al., 2020). Generally, smallholder farmers consume a significant amount of what they produce; therefore, increasing on-farm crops diversity and livestock species is frequently seen as a promising way to improve household dietary diversity (Fanzo et al., 2013; Jones et al., 2014; Powell et al., 2015; Jones, 2016; Sekabira and Nalunga, 2020). Moreover, dietary diversity is often used as a proxy to indicate an individual's broader nutritional status because diverse foods facilitate the balanced intake of all essential nutrients (Webb, 2014). Besides, the corpus of empirical studies acknowledged that increased farm production diversity had a positive influence on dietary diversity; however, it lacks discussion of scale and environmental aspects (Fanzo et al., 2013; Jones et al., 2014; Pellegrini and Tasciotti, 2014; Dillon et al., 2015; Powell et al., 2015; Sibhatu et al., 2015; Snapp and Fisher, 2015; Hirvonen and Hoddinott, 2017; Koppmair et al., 2017; Sibhatu and Qaim, 2018; Zanello et al., 2019). Nonetheless, for smallholders in developing countries like India, where the average landholding is 1.09 ha with varied weather conditions, encouraging smallholders to increase crop diversification may have adverse effects, mainly when crop diversity is already high. It may expose smallholders to the risk of losing benefits from specialized and economically viable crops and gaining a competitive advantage (Sibhatu and Qaim, 2018). In addition, in developing countries, smallholders have limited access to technologies, prevailing diverse agro-climatic and soil biophysical conditions, and scattered markets across the geographical area hinder households from diversifying farm production (Hirvonen and Hoddinott, 2017).

Conversely, recent empirical studies have highlighted the relative importance of markets for farm household dietary diversity and reported that markets access is more critical for farm household's dietary diversity than subsistence production (Luckett et al., 2015; Sibhatu et al., 2015; Jones, 2016; Lenjiso et al., 2016; Qaim et al., 2016; Koppmair et al., 2017; Ludwig, 2018; Sibhatu and Qaim, 2018; Mulenga et al., 2021). Further, A recent systematic literature review on the interplay between food market access and farm household dietary diversity in low and middle-income countries by Nandi et al. (2021), after screening 786 articles, revealed that the majority of the studies highlighted that market access improves dietary diversity. However, none of the studies reviewed have addressed how diverse foods are sourced from markets and on-farms contributing to farm household diet. Besides, most studies considered distance the major proxy indicator (e.g., self-reported travel time, distance to the nearest market, time taken, and cost to reach the nearest market). This proxy indicator may not accurately measure the construct of market access and may not necessarily equate to market participation and food diversity. Besides, distance to the market does not change with the season, while the availability of food changes on-farm and on the market. Therefore, we argue that mere market access does not guarantee the diverse food available to purchase because the type of market (input, output, or consumer market) individual accessing and market food diversity in specific markets play an important role in impacting farm households' diet. Similarly, Mulenga et al. (2021) suggested that future studies be conducted to understand how much food consumed from a particular food group was purchased and what proportion was from the household's production contributing to household dietary diversity to understand the relative importance of on-farm production and market purchases in a given context. Therefore, based on the research gaps identified in the previous literature, we analyzed the relative importance of farm production diversity and market access on farm household dietary diversity by estimating how much food consumed from a particular food group was purchased and what proportion was from own production in the context intensive farming system.

Objective of the Study

Given the scant evidence on this topic and the growing cognizance that dependency on markets satisfy the demand for nutritious non-staple foods even by very poor and remote rural households warrants a detailed understanding of rural markets. Our research questions mainly ask how the rural markets vary in their food diversity, frequency, how rural markets are associated with diets, and their relative importance in household diet and the local production system.

With this backdrop, the present study aims to examine the

1. Relative importance of rural markets food diversity and farm production diversity in contributing to farm household's diet by estimating how much food consumed from a particular food group was purchased and what proportion was from own production.
2. Whether the rural market food diversity is complementary or substitute to on-farm production diversity in contributing "household's" diet.
3. To examine farm production patterns, the rural market food diversity, and seasonality of foods available in the market potentially affect the rural "household's" diet.

To the best of our knowledge, this article is the first to address above mentioned questions, specifically in the Indian context. We employed a novel but highly replicable community and market survey using the mixed method in conjunction with key informant interviews to explore the characteristics of rural markets and their contribution to the household's diet. The results will help policymakers and development practitioners to design successful policies and programs.

Rural Markets, Market Food Diversity, and Their Role

"The soul of India lives in its villages"—Mahatma Gandhi.

India is a land of villages; nearly 68% of the population lives in 640,867 villages (Census, 2011). There are 47,000 periodic markets in India, which will play a significant role in the future as 58% of the rural consumers prefer to buy from periodic markets despite the product being available in the neighborhood stores in the villages (Kashyap, 2016; Velayudhan, 2016). A periodic market is an informal marketplace in the village, and such markets in various towns are as old as the settlement (Satyam, 2018). Some of the markets are located in the center of the community, in prominent places, and in nearby villages. The role of rural markets is very significant for the economic activities among rural dwellers, and largely rural people depend upon periodic markets for the sale and purchase of agricultural commodities. Rural markets play a vital role in supplying diverse diets to farm households, particularly smallholder farmers, due to stronger market linkage (Gupta et al., 2020). Various studies highlighted that rural market linkages enable farmers to consume a diverse diet through demand and supply. On the supply side, markets can make diverse and nutritious foods available to rural households across the seasons (Snapp and Fisher, 2015; Nandi et al., 2021). While on the demand side, increased income through commercial production of a few crops and by selling surplus commodities in the market may raise their income and demand for more diverse foods (Koppmair et al., 2017).

The rest of the article is organized as follows. First, the methods, including study setting, study design, sampling strategy, data collection, analysis, and measurements of different indicators, are presented in Section Methodology. Then, Section Results and Discussion presents and discusses the analysis results, while Section 4 provides the conclusions and implications for policy, followed by study limitations and future research.

METHODOLOGY

Study Setting

This study is "part of a larger interdisciplinary research project, 'Transforming India's Green Revolution by Research and Empowerment for Sustainable Food Supplies (TIGR2ESS),'" conducted in multiple locations across different states in India. It is conducted at the research site representing semi-arid agro-ecology from the south Indian state of Telangana, which has 46,531 irrigation tanks out of a total of 556,000 tanks in India (Kumar et al., 2016). A paddy is being cultivated in 44 million hectares in India, resulting in overexploitation of groundwater resources, particularly in the northwest and some parts of South India. Besides, crop diversity in these regions has significantly declined due to an intensive production system where paddy or wheat is being grown intensively in large areas. Our research site represents three villages, namely Katakshapur, House Buzurg, and Neerukulla, under Atmakur Mandal[1] of Warangal Rural district in the Telangana state of India. These villages were randomly selected. These are the village's representative of larger regions with a market-oriented production system where a paddy is being grown intensively in large areas using tank irrigation. Due to decreased on-farm crop diversity in the selected villages, farm households depend on the local village markets for their diverse food needs. The selected villages are around 30 km away from the city of Warangal and are characterized by local periodical markets and Kirana stores. The Telangana state was a part of Andhra Pradesh state until it was separated in 2014, and Hyderabad City is the capital of Telangana state. The Warangal district is located 150 km from the metropolitan city of Hyderabad. More than 93% of the population live in rural areas in the district, agriculture is the main source of livelihood, and the literacy rate is 62.39–65.97% among males and 41.69–46.2% among females (Census, 2011). Map showing the research site as shown in **Figure 1**.

The characteristics of the study villages are mentioned in **Table 1**.

The villages in Warangal rural district are undergoing rapid transformation from subsistence farming to commercial farming systems due to improved access to water for irrigation, improved agricultural markets, and food processing units in the vicinity that create demand for agricultural outputs such as paddy and maize from the local farmers. Similarly, crop diversity has declined over time from multiple diverse subsistence cropping systems to more market-oriented commercial crops such as paddy, cotton, maize, chili, and turmeric in the district. The nearest city is Warangal, where major agricultural markets (output, input, and consumer), including government's Agricultural Produce Marketing Committee (APMC) and National Electronic Market (eNAM) are situated. The villages have relatively better connectivity with Warangal near the city, and the roads are good. The majority of the farmers sell their farm outputs, mainly paddy, cotton, maize, turmeric, and chili in the Warangal APMC market. Farmers have to collectively depend on their own transport or rent a truck to transport their commodities to the market during harvest season. While farmers have to depend on local markets to buy daily food needs, however market day happens only once a week, accessing the urban daily market is a challenge due to the distance factor.

Study Design

The study adopted a mixed method of research approach. The mixed-method is an emerging methodology in social sciences that sees through multiple lenses to understand the complex research questions in the field. The mixed research method integrates quantitative and qualitative data within single research, permitting a synergistic use of data than using separate quantitative and qualitative data use and analysis (Creswell, 1999; Johnson and Onwuegbuzie, 2004; Brannen et al., 2005; Morse, 2016). To understand the changing cropping pattern, crop diversity, and crop area in the study region, we employed the quantitative data at the district level from 1966 to 2015 which is collected from ICRISAT District Level Database (ICRISAT - DLD, 2020). In addition, data about household food consumption, health, and nutrition-related information is collected from district-level offices. The qualitative household and community level information is collected through the Focus Group Discussions (FGDs). Besides, information about market food diversity and the seasonality of food available in the markets are collected from traders, agents, and farmers through Key Informant Interviews (KII). In addition, physical visits to the markets were made (Ambikapathi et al., 2019), to understand the market food diversity seasonality of food availability in specific village markets. We hoped that both quantitative and qualitative findings could be used to interpret, analyze, suggest, and guide

[1] Manda is administrative unit, which is formed with group of villages.

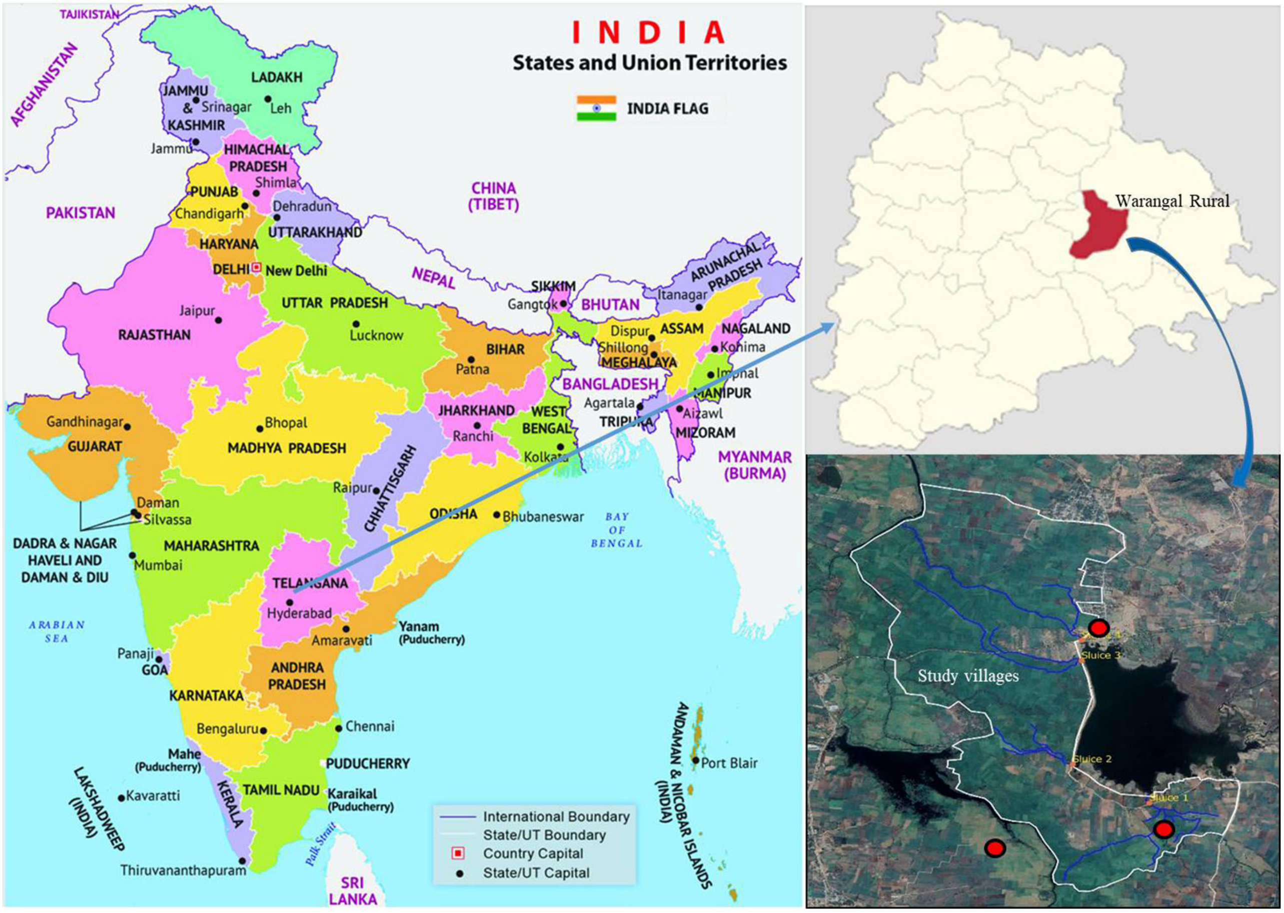

FIGURE 1 | Map showing the research site (map not to scale), red dots in the google map are villages in the Warangal (R) district of Telangana state. Source: Adapted from: mapsofindia.com.

TABLE 1 | Characteristics of study villages.

Village	Dependency on farming	Population		Landholding* (ha)				Literacy rate	
		Male	Female	Marginal	Small	Medium	Large	Male	Female
Neerukulla	96%	2101	2124	798 (81%)	124	63	0	65.97	46.28
Katakhapur	92%	444	451	214 (73.3%)	78	0	0	62.39	41.69
House Buzurg	97%	688	671	226 (67%)	106	7	0	54.07	39.34

Marginal up to 1 ha, Small 1–2 ha, Medium 2–10 ha, and large >10 ha. Source: village revenue office.

appropriate interventions at the community and next level of the study sites.

Sampling and Data Collection

The field enumerators are recruited to collect the qualitative data, and trained on research protocol to ensure their understanding of the broader objectives of the study. The recruited field enumerators were familiar with Telugu local language to conduct the FGDs and KIIs in the local language. The respondents were carefully selected by following stratified purposive random sampling from the list of households obtained from each village revenue office. The FGDs were held separately with male and female groups. While doing so, care was taken to ensure the fair representation of households covering all caste and classes existing in the village. Each FGD group ranged from 8 to 10 respondents. To achieve saturation of information on a specific theme, we continued conducting the FGDs until we reached a point where we had a range of opinions and no new information was collected. In addition, KIIs are conducted by visiting respective "villages" periodical markets. After Focus

TABLE 2 | Sources of data.

Qualitative data	Quantitative data
• Total of 34 sex-segregated focus group discussions with 254 households (119 males, 135 female). • Physical visits to local periodical markets • Seasonal food availability in the local markets	• Household-level dietary diversity. • Six Key Informant Interviews (KIIs) • District-level data from 1966 till 2015 about crop diversity. • Market Food Diversity (MFD)

Group Discussions (FGDs), the individual participants also answered the survey questions related to household consumption patterns. The details about the sample and source of information are mentioned in **Table 2**.

Data Analysis

This qualitative research is grounded in interpretative approaches, and each theme-based analysis was inductively and deductively identified from the qualitative data. Inducting coding was made based on the grounded theory technique, including line-to-line analysis and the comparative method. All the transcripts were coded independently by the lead author and co-lead. Further, the discussion was held with other team members to interpret the results. The data analysis from the FGDs and KIIs with respondents and videos and audios were zed using content analysis and the software RQDA for Qualitative Data Analysis, mainly used to systematically structure large text information (Chandra and Liang, 2016). For all the qualitative data from the project involving responses to different research questions, RQDA was used to systematically cluster responses to open and to probe questions into meaningful categories. We used only relevant qualitative information specific to this study from the entire qualitative data. Besides, quantitative data is analyzed using MS excel to arrive at the participants' Food Consumption Score (FCS), Household Dietary Diversity Score (HDDS), and socioeconomic profile.

Measurements

We employed the FCS and HDDS, which are important qualitative measures of household food consumption and are cost-effective and less time-consuming compared to quantitative dietary intake methods (Kennedy et al., 2010). These are the proxy measures of household dietary intake. The Food and Agriculture Organization (FAO) and the World Food Program (WFP) use information about dietary diversity as one element to inform food security studies. Albeit, the FAO uses a 1-day HDDS based on the Food and Nutrition Technical Assistance Project guidelines, whereas the WFP uses an FCS (Swindale and Bilinsky, 2006). Both HDDS and FCS have been validated in different countries as a proxy measure of caloric intake (Wiesmann et al., 2009; Kennedy et al., 2010; Hussein et al., 2018). The validation studies have shown that FCS and HDDS are both related to caloric intake and each other. The data gathered using either measure are most useful for application within a given agro-ecological zone or country (Kennedy et al., 2010).

Food Consumption Score (FCS)

Food Consumption Score is an index developed by WFP in 1996, and it is a proxy indicator of household caloric availability. The FCS aggregates household-level information on the diversity and frequency of food groups consumed during the previous seven days, which is then weighted according to the relative nutritional value of the consumed groups (World Food Program, 2008). FCS consider eight food groups and each food group consumed receives a weight from 0.5 to 4 (cereals, tubers, and root crops = 2; meat and fish = 4; milk = 4; oil/fats = 0.5; fruit = 1; vegetable = 1, pulses = 3, sugar = 0.5) and condiments are not counted in FCS (Kennedy et al., 2010). The typical cut-off scores were ≤ 21 (poor), borderline (21.5–35), and acceptable (>35).

To construct the FCS, we used information on the household's food consumption and the frequency of specific food groups/items during the previous 7 days. The food items were then grouped into eight specific food groups. Any frequency values over seven are capped at seven. Finally, each food group was multiplied by a food group weight, and the sum of the weighted food group score is calculated to arrive at Food Consumption Score (FCS).

Household Dietary Diversity Score (HDDS)

Household Dietary Diversity Scores are calculated by summing the number of food groups consumed in the household or by the individual respondent over the 24-h recall period. More diversified households' diet is correlated with protein and caloric adequacy (Swindale and Bilinsky, 2006). We use a number of food groups consumed by the farm households over the last 24 h to create HDDS as a proxy for household diet quality, and it is validated as a measure of nutrient adequacy and food security using 24-h recall periods for women and children (Verger et al., 2019; Nandi et al., 2021). To measure HDD, we categorized reported food items into food groups to align as closely as possible with the FAO guidelines (Kennedy et al., 2011). The 12 food groups included are namely cereals; white tubers and roots; legumes, nuts, and seeds; vegetables; fruits; meat; eggs; fish and fish products; milk and milk products; sweets and sugars; oils and fats; and spices, condiments, and beverages are used to calculate the HDDS indicator. Each food group is assigned a score of 1 if consumed or 0 if not consumed. The household score ranges from 0-to 12, and it is equal to the total number of food groups consumed by the household. The aggregated food consumption index measures the sum of groups of foods consumed within a household, and it reflects the dietary quality of foods available to households and is used as a household nutrition security indicator (Swindale and Bilinsky, 2006). HDDS ≤ 3 is considered a low dietary diversity group, with between four to six as medium and ≥ 7 as high diversity score category. We have considered ≤ 3 as a low dietary score because, as a general rule, consuming four food groups over 24 h is considered good dietary diversity (Kennedy et al., 2011). However, there is no international consensus on which cut-off values to use (Vanessa Cordero-Ahiman et al., 2017).

$$HDDS = SUM\ (A+B+C+D+E+F+G+H+I+J+K+L)$$

The average household dietary diversity score for the target population can be calculated as follows:

$$\frac{Sum\ (HDDS)}{Total\ number\ of\ households\ surveyed}$$

Crop Diversity Index (CDI)

Crop diversity refers to growing several crops in a year in the given landholding. There are different methods to measure crop diversification, and the important one is Herfindahl-Hirschman Index (HHI). HHI is calculated by taking the sum of squares of acreage proportion of each to the total cropped area (Sharma, 2017).

$$HHI = \sum_{i=1}^{N} Pi^2$$

Where,

N = The total number of crops.

Pi = is the proportion of area under ith i = 1 i = 1 crop to total cropped and Ai = is the actual under ith crop.

The index is defined as a sum of squares of all "n" proportions and is a measure of concentration.

The value of HHI ranges between 0 and 1, where zero indicates perfect diversification and one indicates perfect specialization. The value of HHI approaches zero as "N" becomes large and takes value one when only one crop is cultivated (Sharma, 2017). The HHI is categorized into three levels: HHI Below 0.1 is less diversified, 0.1–0.18 is moderately diversified, and HHI above 0.18 is highly diversified.

Market Food Diversity/Market Food Availability

The most commonly used variables for market access indicator in the literature is self-reported to nearest markets, the existence of a market in the village, roads, and ownership of vehicles (Sibhatu et al., 2015; Snapp and Fisher, 2015; Nandi et al., 2021), the share of farm produce sold to proxy for market access at household level (Jones, 2016). In our study, we use a similar measure to access the market, but it is market food diversity to measure the diversity of food available in the specific market across the season, and on the consumption side, the share of purchased foods from the local markets in household diet during the last seven days.

Market Food Diversity (MFD) is defined as the availability of foods and food groups across the seasons in a given market. MFD is a new definition that was recently used by Ambikapathi et al. (2019), and a similar definition of market food availability by Zanello et al. (2019). The MFD information can be collected using the information gathered from traders (from their book transactions), shopkeepers, other key informants, and visiting the target markets. Conversely, in the previous literature, mainly market access is used as an indicator to study the role of markets in household diet quality. But mere access to the market cannot guarantee the availability of diverse foods (Nandi et al., 2021). It is reported from several studies that food crop and livestock diversities have a positive relation with dietary diversity. In our study, we collected MFD information by interviewing regular vendors and traders in the market and also by visiting village markets. There are two key informants for each village. The information collected is about availability of different food items belonging to different food categories, price, abundancy of food available, frequency of market and seasonal variability of specific foods in the village market. Based on the availability of 32 foods, 7–10 food groups are created to form monthly market food diversity for individual village. The 32 foods are as mentioned in **Figure 4**.

Seasonality

In India, there are three cropping seasons, namely Kharif (June-November), Rabi (October-March), and Zaid (between Kharif and Rabi). Generally, the production of the major staple come from the Kharif season, whereas pulses and oilseeds are from the Rabi season, including wheat. But due to diverse climatic conditions across the geographical area, there is significant variability in seasonal agricultural patterns in India. As a result of seasonal variability, the availability of foods in the local markets varies. Understanding such variability in a specific geographical area is important due to its potential impact on the diet quality of the population who depend on such a market for their daily consumption. Therefore, there is a need to understand local food availability in the local markets and its impact on the 'household's diet, as such information is missing in the literature in low and middle-income countries. This article advances the literature on the importance of seasonality on market food availability and diversity that potentially affect household dietary diversity. Besides, we assess the role of rural markets' food diversity than the previous literature.

Ethical Approval

Ethical approval was obtained from the Institute Review Board (IRB) for the ethics of ICRISAT (Reference number IEC-ICRISAT/20190818/01).

RESULTS AND DISCUSSION

Descriptive Statistics

The sociodemographic characteristics of the respondents are shown in **Table 3**. Of the 254 total respondents who participated in the FGDs, 47% were males with an average age of 47 years, whereas 53% of the respondents were females with an average age of 41 years. The majority (53%) of the households belong to Backward Class (BC), and 20% are Scheduled Caste (SC), 17% Other Caste (OC), and 9% belong to Scheduled Tribe (ST). Thirty-eight percent of the respondents were illiterate without any formal education, and only 15% had formal education, graduated and, pursued higher studies. The remaining 40% studied middle school to higher secondary. The majority (89%) of the households are landed, involved in farming, and mainly belong to OC and BC caste, 11% were landless and mainly belong to SC and BC caste, and the majority of them are farm laborers. The health and nutritional data of study villages are not part of our survey. However, to provide an overview of the district's health and nutritional status, district-level information is provided in **Annexure 1**.

TABLE 3 | Sociodemographic characteristics of the survey respondents.

	Variable	FGDs (*N* = 254)
Gender	Male	119 (47%)
	Female	135 (53%)
Caste	OC*	42 (17%)
	BC*	135 (53%)
	SC*	52 (20%)
	ST*	24 (9%)
Av. Age	Male	46.67
	Female	41.13
Education	Illiterate	96 (38%)
	Up to middle school (1- 8th std)	63 (25%)
	High and higher sec (9–12 std)	58 (15%)
	Under graduation and above	37 (15%)
Occupation	Farming	227 (89%)
	Farm labor	27 (11%)
Land status	Landed	227 (89%)
	Landless	27 (11%)

**OC, Others; BC, Backward Class; SC, Scheduled Caste; ST, Scheduled Tribe.*
Source: Author's compilation based on survey data.

Cropping Pattern in the Study Villages

More than 94% of the population depends on agriculture for their livelihood in the study villages. Paddy occupies the 'lion's share in the cultivable area, and it is the main crop during Kharif season, followed by cotton, maize, groundnut, chilies, and other few crops in less area. Maize is not consumed locally, and it is grown for commercial purposes (processing and poultry feed). Due to the well-established government-supported Agricultural Produce Market Committee (APMC), rice mills, and other private buyers within the village vicinity and farmers generally "don't face any problem selling their produce." Farmers often have prior contact with potential buyers through their inputs and credit transactions. The free electricity, access to tank irrigation, MSP, and subsidized inputs are major push factors for farmers bringing more area under paddy. In particular, for paddy, access to water from the local village tanks helps farmers to grow two paddy crops in a year (both in Rabi and *Kharif*). Besides, enabling environmental factors such as local markets (APMC), village-level procurement ers (under MSP), rice mills, poultry feed factories, and local production ecosystems are favorable for farmers to grow paddy and maize. Besides, access to high yielding varieties (HYVs) and improved inputs such as seeds, fertilizers, and plant protection chemicals helped increase the production and productivity of paddy and maize crops in the region over the period (**Figure 2**).

On-Farm Crop Diversification and Farm Income

The cropping system in the study region has shifted from subsistence farming to a commercial and market-oriented production system, mainly due to easy access to irrigation water and a better market for agricultural commodities. We analyzed crop diversification from 1966 to 2015 using longitudinal data about crops in the Warangal rural district of Telangana state using Hirscman - Herfindahl Index (HHI) method to measure the crop diversity. The results revealed that the crop diversity had declined significantly from a highly diverse (HHI >0.18) cropping system to a very less diversified (HHI < 0.1) cropping system between 1966 to 2015 (**Annexure 2**). From 1966 to 1975, the 10 "years" average HHI was 0.20, which is a highly diverse cropping system, and it continued till the year 1984 with HHI ranging from 0.20 to 0.18. After the year 1985 onwards till the year 2010, the HHI was moderate, with the HHI values ranging from 0.16 to 0.11. However, HHI values declined below 0.10 from 2011 until 2015, indicating that crop diversity has significantly declined in the district during recent years. This clearly shows a rapid decline in the district's crop diversity, which severely impacts natural resources, ecology, household health, and nutrition. The impact of farm production diversity on-farm household's nutritional outcomes is well-established in the literature (Pellegrini and Tasciotti, 2014; Powell et al., 2015; Sibhatu et al., 2015; Snapp and Fisher, 2015; Hirvonen and Hoddinott, 2017; Koppmair et al., 2017; Sibhatu and Qaim, 2018; Zanello et al., 2019) highlighting decreasing farm production diversity directly impact household nutrition in the absence of access to the market. The rapid decline in crop diversity in the region is due to the increased number of farmers shifting from diverse cropping patterns to more commercial crops such as paddy, cotton, maize, etc. It is observed from the detailed analysis that some of the crops, particularly wheat, sorghum, pearl millet, finger millet, minor pulses, and oilseeds, have lost their place in the total crop area, and the area is being replaced with cash crops, mainly cotton (**Annexure 3**). The cereal crops occupied the lion's share in the total cropped area in the district (**Annexure 4**).

Focus Group Discussions also revealed similar results at the village level in the study area. Some of the FGDs participant's responses related to changing crop diversification in the study area are mentioned below,

> "*We used to grow variety of crops including fruits, vegetables, millets etc. 20–40 years back. The majority in our villages grow paddy, cotton, and maize crops due to access to water from tanks, better price, assured markets, and easy to cultivate, particularly paddy*" (Male, Landed, FGDs, House Buzurg).

Another FGD participant reported,

> *"[. . .] In our village majority grow paddy with tank water, we also grow watermelon, cucumber etc. in a small patch of our land along with paddy crop, but monkeys and wild boar menace is severe that destroy fruits and vegetable crops, we must monitor day and night if we plant such crops. Therefore, we don't prefer to grow other than paddy, maize, and cotton*" (Female, landed, FGD, Neerukulla).

Key informant interviews also revealed similar views on changing crop diversity. One key respondent said:

> *"[. . .] During our childhood days, my parents used to grow different pulses, millets, vegetables, fruit crops along with paddy crops or during the rabi season, but nowdays [sic], we can see only two to*

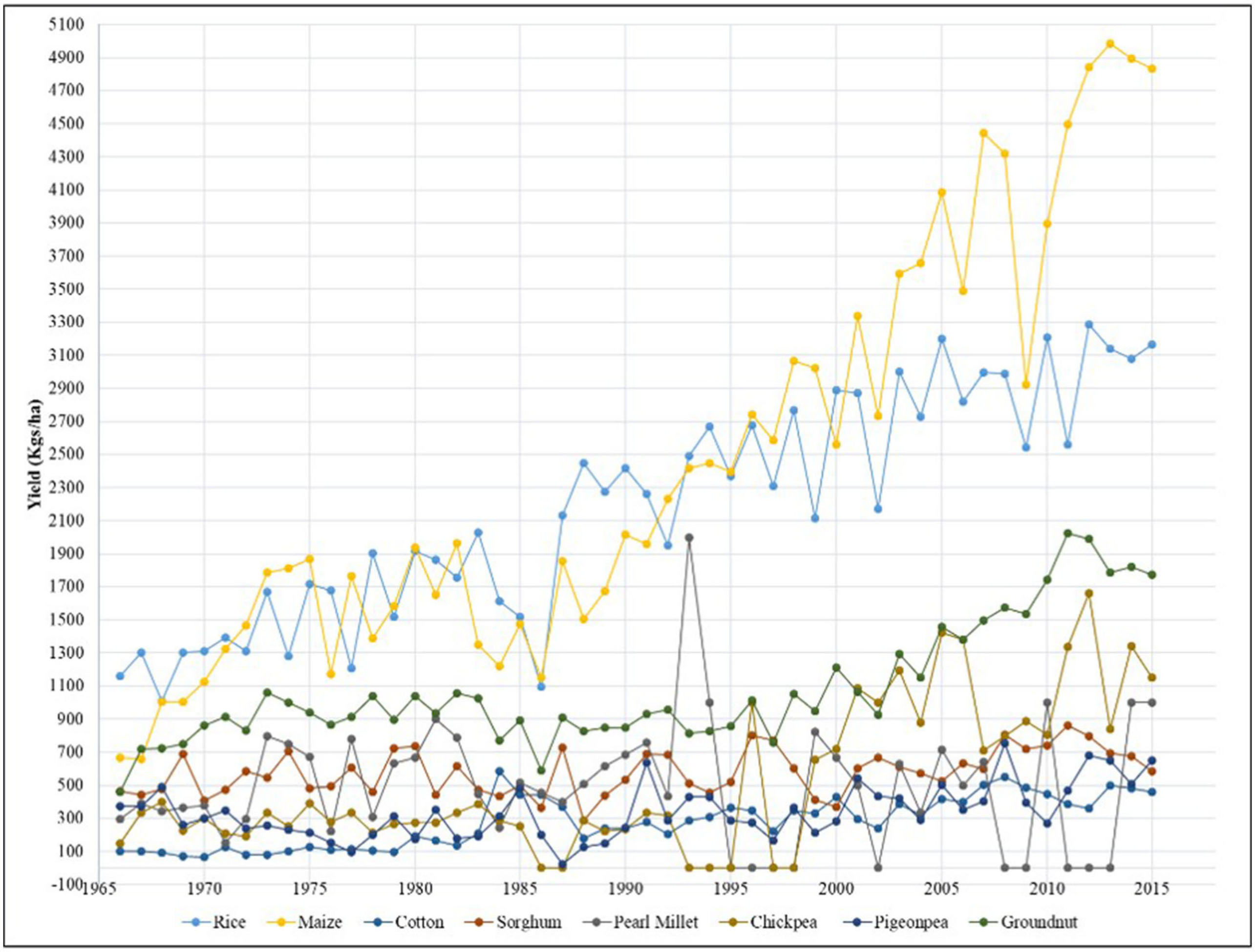

FIGURE 2 | Yield of paddy, maize, and other major crops over time in the study region.

> *three crops in surrounding villages*" (Male, KII, Katakshapur, Age 56 years).

Another key informant mentioned,

> *"During 15–20 years before, the study villages were growing diversified crops such as paddy, wheat, sorghum, pearl millets, finger millets, minor pulses, oilseeds, fruits, vegetables, chili, and other crops. In addition, the majority of the households in the villages had at least 2–4 milk animals, and every household used to sell surplus milk to the city. Then, however, the majority of the crops disappeared, and paddy became a major food crop. As a result, the majority of the households buy milk packets from outside*" (Male, KII, landed, Sarpanch, Age 52, Neerukulla).

Due to increased market-oriented commercial production of crops, mainly paddy, maize, and cotton in the study region, farmers' income has improved over time. This is due to increased production, productivity, and assured government's Minimum Support Price (MSP) for the crops mentioned. As a result, the farmers in the study villages received an average Rs. 1,800/quintal of paddy, Rs. 5,800/quintal for cotton, and Rs. 1,400 for maize under MSP during 2020.

Relative Importance of On-Farm Production and Rural Markets in Contributing Farm "Household's" Diet

Similar to the majority of the Indian villages, in our study village markets (consumer market) takes place once a week (periodical markets), and rural communities rely on these periodical markets to buy their household food needs (vegetables, fruits, groceries, meat, etc.), which are not grown in their farm. Therefore, we exclusively assessed the relative importance of on-farm production and purchases from consumer markets in contributing to farm 'household's dietary diversity. Based on

TABLE 4 | Descriptive statistics for FCS and HDDS in rural Warangal district.

	Indicators					
	FCS			HDDS		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**
Scores	60.81	6.82	47–67	7.63	2	4–10
Own farm	14 (22.71 %)	0.81	12–14	3.06	1.48	0–6
Market purchase	47 (77.29 %)	6.65	35–58	4.56	1.63	2–8
Women						
Scores	57.38	7.52	49–67	8.25	1.49	6–10
Own farm	13.75 (24%)	0.71	12–14	3.63	1.51	2–6
Market purchase	43.63 (76%)	7.15	35–53	4.63	1.60	3–7
Men						
Scores	64.25	4.03	59–72	7.00	2.33	4–10
Own farm	13.50 (21%)	0.93	12–14	2.50	1.31	0–4
Market purchase	50.75 (79%)	3.81	45–53	4.50	1.77	2–8
Neerukulla	Mean	SD	Range	Mean	SD	Range
Scores	58.4	6.7	49–65	9.8	0.4	9–10
Own farm	14 (24%)	0	14	3.8	1.8	2–6
Market purchase	44.4 (76%)	6.7	35–51	6	1.6	4–8
Katakshapur	Mean	SD	Range	Mean	SD	Range
Scores	64.33	6.35	54–72	7.67	0.82	7–9
Own farm	14 (21.8%)	0	14	3.50	0.84	2–4
Market purchase	50.33 (78.2%)	6.35	40–58	4.17	1.47	4–7
H.Buzurg	Mean	SD	Range	Mean	SD	Range
Scores	59	7.1	47–65	5.4	1.34	4–7
Own farm	12.8 (21.7)	1.1	12–14	1.8	1.1	2–3
Market purchase	46.2 (78.3%)	6.72	35–51	3.6	0.9	2–4

FCS, Food Consumption Score; HDDS, Household Dietary Diversity Score.
Source: Household survey.

the household-level food consumption, we estimated both Food Consumption Score (FCS) and Household Dietary Diversity Score (HDDS) to differentiate the relative importance of on-farm production and market purchases. Besides, gendered and inter-village differences in food consumption from different sources is analyzed. Furthermore, we estimated how many food groups were consumed from their own production and what proportion was purchased (**Table 4**).

From **Table 4**, the mean FCS of the farm households in the study villages is 60.81, which indicates the households are food secure (FCS > 35). The contribution from own production to the total FCS is 22.71%, whereas market purchases contribute 77.29%. Similarly, the mean HDDS is 7.63, which means farm households consume diverse foods representing an average of 7.63 food groups (≥7 as high diversity score category) out of 12 food groups considered. The contribution from own production to the total HDDS is 3.06 (≤3 is considered low dietary diversity), whereas market purchases contributed an average of 4.56 food groups. The detailed analysis of FCS and HDDS (**Supplementary Material 1**) revealed that the farms mainly supplied cereals with an average FCS of 13.63. The average frequency of consumption of cereals per week is 6.81. Whereas market purchases supply pulses, fruits, vegetables, meat, fish, milk, sugar, and cooking oil. The individual FC scores ranged from 0.38 (fruits) to 24.50 (milk), and the weekly average frequency of consumption of individual food groups ranged from 0.40 (fruits) to 6.13 (milk). Though the frequency and FCS of milk is higher, the quantity of milk consumed is meager (only for mixing with tea and preparing curd to eat along with rice). This is one of the limitations of FCS calculation as it considers only the frequency of consumption of a specific food group and does not consider the quantity of food intake. The quantity of milk products consumed may not be adequate to supply the required nutrition to individuals. Further, there is gendered differences observed in FCS and HDDS, with "women's" mean FCS and HDDS being 57.38 and 8.25, respectively. Whereas "men's" FCS and HDDS is 64.25 and 7.0, respectively. The results clearly show there is gendered differences in dietary diversity. The gendered differences may be linked to sociocultural aspects of individuals. Similarly, inter-village differences with FC and HHD scores range from 47 to 67 with a standard deviation of 6.82 for FCS, and 4–10 food groups with a standard deviation of 2 for HDDS are observed. The wide variation is mainly attributed to the level of diverse foods and food groups available in the specific market from which specific village purchases. From **Figure 3**, it is clear that village market 3 has low diversity of foods available as compared to village markets 1 and 2, and from **Table 4**, it is evident that House Buzurg village, which buys foods from the village market 3 has food groups available ranging from 2 to 7, as compared to the other two villages with food groups ranging from 7 to 10. Conversely, the FC scores of House Buzurg village are distant with the other two villages. This is mainly because of the type of food it often purchases (milk, meat, and fish) from village market 3. Further, the detailed analysis of the determining factors for gendered differences in dietary diversity in food sources is scope for future study. Overall, the market purchases significantly contribute to the farm household dietary diversity than on-farm production. The literature confirms the results (Luckett et al., 2015; Qaim et al., 2016; Ludwig, 2018; Mulenga et al., 2021). Further, specific food groups consumed at farm households are assessed to understand the share of own farm and market sources in each food group. The results revealed that 93.75% of the cereals consumed at households are from their own production, whereas 93.75% of oils/fats and spices, and condiments are from local markets. Similarly, the contribution from each source contributing to the farm households' dietary diversity is shown in **Figure 2**. This clearly shows that currently, the market plays a vital role in the food security of households, as it supplies majority of food groups.

Market Food Diversity

We used a new definition of Market Food Diversity (MFD) to characterize rural markets, which was recently used by Ambikapathi et al. (2019), to analyze the access of diverse foods to the farm households. In the literature, the most common indicator that is used for market access is distance to market, however, distance to market cannot guarantee the availability of diverse food in the market. This is a significant point, as it is plausible that the diversity of food available in the markets

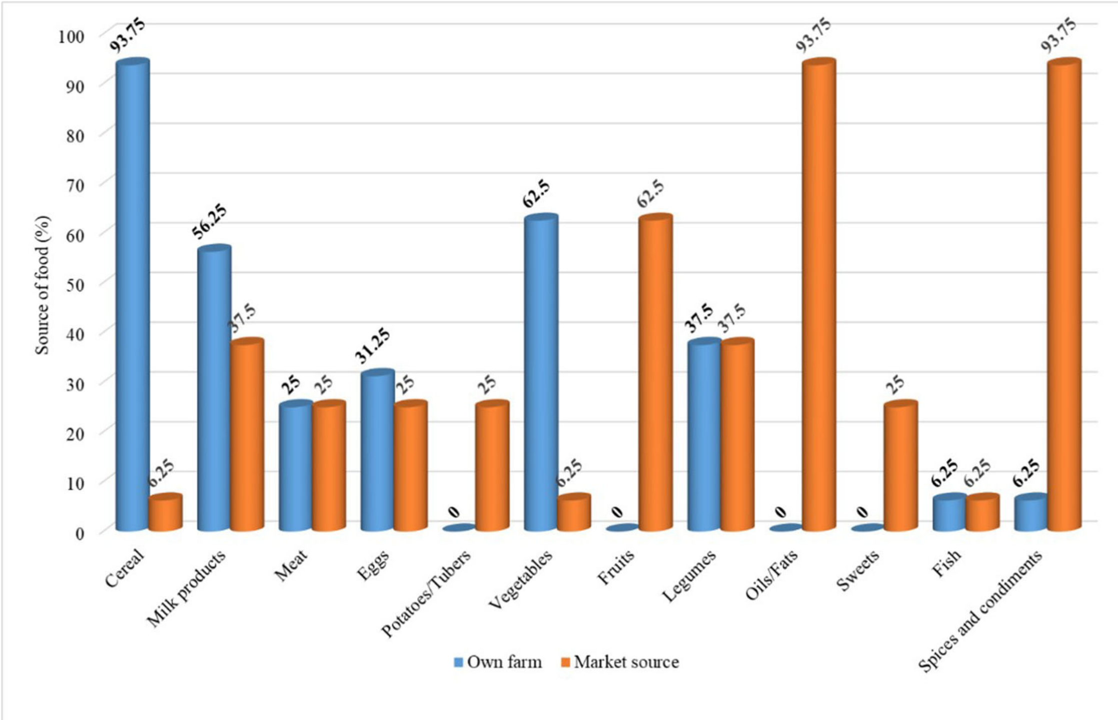

FIGURE 3 | Consumption of each food group sourced from own farms and or local markets among farm households.

is a vital source for a diverse diet for households (in a similar way to the influence that farm production diversity has on dietary diversity). In our study villages, MFD from 7 to 10 food groups comprises 62–65 crop species during June–July 2020. This contrasts with average crop diversity (number of species) in the study villages which was only 10.50, and livestock diversity (species) which was 4.93. It is very clear that the diversity of food available in the market was much higher than own farm production. However, the limitation of the rural markets in the study villages is that the market takes place once a week and households therefore can source their food only once a week. This situation affects the purchase pattern of perishable foods as the majority of the farm households don't have refrigerators. Even the few farmers who have their own refrigerators are not able to use them due to frequent power supply interruptions and even during the summer season, the power supply is restricted to a few hours during the daytime. In conclusion, farm production contributes mainly to caloric intake by supplying mainly cereals (from paddy production), whereas local markets, including the village Kirana shops, supply diverse nutritious foods to the farm households (**Supplementary Material 1**).

Seasonality and Market Food Diversity

Agricultural production, market access, availability of food, and consumption are all potentially highly amenable to seasonal influences, specifically in developing countries (Zanello et al., 2019). From **Table 4**, it is clear that a significant share of food is sourced from local markets by farm households during the time of our field survey. The diversity of food available in the local village markets was collected simultaneously as the household surveys in June and July, allowing seasonal comparison of the availability of diverse food in the local markets. From **Figure 4**, it is evident that local markets are variable in availability, diversity, and abundance of foods belonging to different food groups available in the local markets differ across the seasons, and it potentially affects the farm household's dietary diversity in the absence of own farm production. Further, of the three study villages' local markets surveyed, market 1 is the most diverse, and the village Neerukulla has access to it, while market 2 has intermediate diversity, seasonal fluctuations, and the village Katakshapur has access to it. Conversely, village market 3 (**Figure 4**) has low diversity and seasonal fluctuation, the House Buzurg village has access to it. The village markets 2 and 3 have intermediate to low diversity and seasonal fluctuations, and these two markets fail to supply a wide range of foods during the lean season, particularly pulses, dark green leafy vegetables, fruits, organ meat, flesh meat, fish, and fish milk products. Since the local crop production is significantly skewed toward staple (Rice, Maize) crops, there is no demand for such products in the local markets, and therefore, such products are not abundantly available in the markets. Our results are consistent with the literature, where we observed that seasonality and agro-ecological zone play a role in market food diversity in Ethiopia (Ambikapathi et al., 2019) and the importance of markets to dietary diversity in Afghanistan (Zanello et al., 2019). Similarly, in their study in Ethiopia, Sibhatu and Qaim (2017) observed

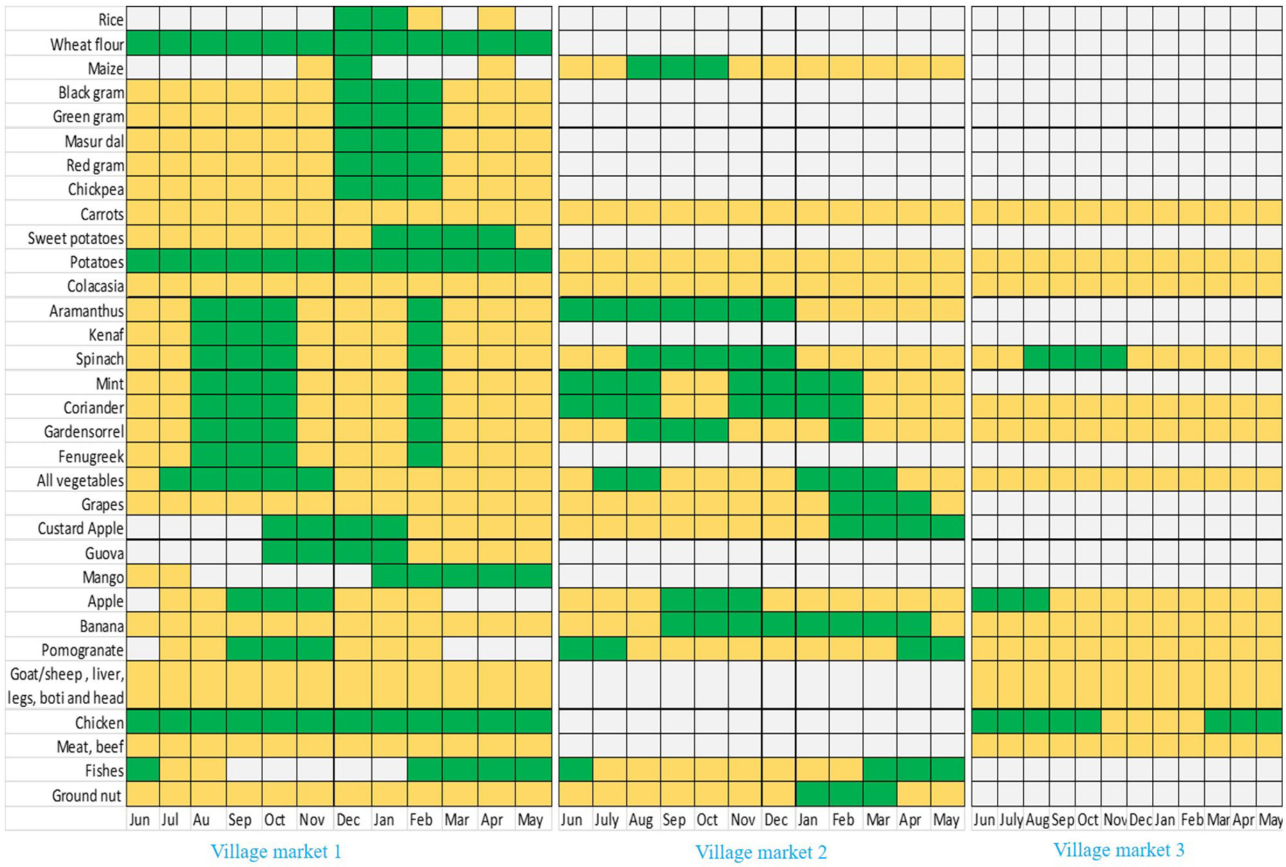

FIGURE 4 | Seasonal food availability in the local village markets (three study villages). Green color indicates abundant availability of selected foods, yellow color indicates moderate availability, and blanks represents no availability in the specific market during specific months.

that food sourced from markets has a larger contribution to farm households' dietary diversity than own production across the seasons.

Figure 4 illustrates the availability of each of the 32 foods at the markets found in each village market across 12 months. Overall, **Figure 3** shows poor access to pulses, fruits, vegetables, eggs, and meat in the local markets. Foods like dark leafy vegetables, potatoes, grapes, custard apple, meat, and fish show strong seasonality. The promotion of such products might improve consumption at farm household's level.

In addition to the seasonal availability of diverse foods, the frequency of market day occurrence in the study villages potentially affect the farm 'household's diet, as most of the local village markets happen once a week and buying and storing perishables such as fruits, vegetables, and milk products are difficult as most of the households lack storage facilities such a refrigerator.

CONCLUDING REMARKS

Majority of the undernourished people live in rural Asia and Africa, and many of them are smallholder farm households that largely depend on agriculture for their livelihoods. A poor-quality diet is one of the vital contributing factors to undernutrition. This is particularly true in India. The rapid transformation of agricultural production systems from subsistence to commercial and market-oriented production augmented the effect due to declining farm production diversities. Generally, smallholder farmers consume a significant share of what they produce; increasing market-oriented production systems resulting in a decline in crop and livestock diversities, ultimately impacting household dietary diversity. Several recent studies consistently highlighted that farm production diversity directly influences the dietary diversity of farm households. Due to decreased on-farm production diversities, farm households rely on local markets (if accessible) to buy diverse food items from the increased income from the sale of crops produced through specialization. Our study intends to answer the relative importance of rural markets access (MFD) and farm production diversity in contributing to farm households' diet by estimating how much food consumed from a particular food group was purchased and what proportion was from own production in the context of a market-oriented production system. To uncover, whether the rural market food diversity and farm production diversity are complement or substitutes each other in contributing a farm household's dietary diversity. Besides, to examine the seasonality of foods available in the market that potentially affects the rural "household's" diet, if the foods availability varies with seasons.

The time-series data from 1966 to 2015 about crop diversity measured using Hirscman - Herfindahl Index (HHI) method indicates that the cropping system has significantly transformed from a subsistence to a market-oriented commercial production system. This transformation has brought a significant area under commercial and market-oriented production where only a few staple crops (paddy, maize) and commercial crops (Cotton and turmeric) are being grown compared to diverse cropping systems before. It is observed from the detailed analysis that some of the crops, particularly wheat, sorghum, pearl millet, finger millet, minor pulses, and oilseeds, have lost their place in the total crop area, and the area is being replaced with cash crops. As a result, the crop diversity has declined significantly from a highly diverse (HHI > 0.18) cropping system to a very less diversified (HHI < 0.1) cropping system over the years. The prevailing cropping system is supplying only starchy staples to the household diet. However, increased income from commercial crop production enabled households to buy diverse foods from the local markets which are not grown in their own farm. Foods purchased from markets representing diverse food groups and are supplementary to the foods produced on-farm. The increase in food group consumption from the local village markets compensates for the decline in food group consumption from own production. This clearly shows that the market currently plays a vital role in the households' food security, as it supplies a majority of food groups. The contribution from own production to the total FCS is nearly 23%, whereas market purchases contribute around 77%. The frequency of local village markets is an important constraint for regular access to diverse foods from the market as these village markets are periodical in nature and happen only once a week. Besides, the absence of storage (refrigerators) at farm households makes perishable food unavailable for consumption regularly. The food diversity and abundance of availability vary across the market and seasons in the study villages, potentially impacting the household's diet. The majority of the previous studies used market access as a proxy by actual reliance on markets for consumption at the village level. Our study shows that mere market access does not guarantee the availability of nutritious food in the specific market as market food diversity varies from market to market and season.

Here, our argument is not for or against the increase in on-farm diversity or market access (market food diversities), which directly influences household nutrition as both have pros and cons of their own (Nandi et al., 2021). However, how to improve dietary quality while maintaining crop diversity, increase in income through commercial crops, and access to diverse foods from local markets by bringing synergy through optimizing benefits from each to address the nutritional issues in a specific context. The effects of changes in on-farm production diversity and market access (market food diversity) differ for different food groups as each food group has its unique potential to contribute to diet quality. The effect of on-farm production may be more critical for some food groups, and market access may be for other food groups.

Based on our results, the following policy suggestions target farm households to improve nutrition in rural areas, specifically in the highly market-oriented/commercial production system.

- Particularly in the context where crop production is less diversified (HHI[2] < 0.1), encouraging diverse crop production will help in better diet quality, manage price risk, and also help in harnessing the multi-functional nature of diversification. However, increasing crop diversification is easier said than done in the commercial production system where profit is at the center, but nuanced policies, including crop diversification compensation/incentive package for identified regions, might be necessary to support these multiple objectives.
- Local markets play a vital role in supplying diverse foods to the people. Therefore, there is a need for strengthening rural markets through developing infrastructure facilities (Ex. cold storage) to make it accessible for rural households to purchase diverse food across the seasons. It is to mention here that the promotion of local markets is not against the on-farm production diversity it is complementary to on-farm production diversity in improving the quality of farm household diets.
- Considering the unorganized retail sector and with the advent of new farm policies in India, the government may also think about incentives to retailers to set up retail outlets in the suburbs (*mandals*), connecting rural areas with mini supermarkets (like the hub and spoke model) and also subsidizing nutritious and/or biofortified foods in rural areas based on the context.
- In addition to the specific policy suggestions proposed based on the field study, a few additional policies are promising that are drawn based on the KII, context, and broader policies in the country. Many public, private, international developmental organizations, and civil societies working in agriculture, marketing, extension, nutrition, health, water, sanitation, and education are working independently without functional coordination among themselves at field level. Therefore, synergy is needed at different levels (policy, planning, and execution).

Study Limitations and Future Research

This study has a few limitations. First, the survey was not designed to understand the individual dietary habit but to look primarily at the household level, and foods share from both on-farm and market purchases at households. This makes the information less than ideal for analyzing the diet intake at an individual level. Furthermore, this is not ideal for assessing the consumption information, as there is one respondent in a household and is unlikely to recall all the household members during the last seven days. This may pose a problem if the respondent overstates or understates consumption for some family members.

Nevertheless, they have helpful information about the overall pattern of food consumption (food groups). Secondly, the use of

[2]The HHI is categorized into three-level, HHI Below.1 is less diversified, 1–0.18 is moderately diversified while HHI above 0.18 is highly diversified.

FCS and HDDS to measure household nutrition is not without their limitations as neither of these indicators has been validated as a proxy for micronutrient adequacy. Thirdly, our results on on-farm diversity, market food diversity, and associated effects on a household's diet need to be considered cautiously as they are context-specific while generalizing the results.

The study identified research gaps for future research. First, further research exploring the preferences of households about what to produce on-farm desired purchases, and sell in a different context is needed as an entry point for improving household food choices among farm households. Second, results highlight that market purchases contribute more to household diet than own farm production. However, the literature is meager about how safe and hygienic foods are sourced from markets compared to their "farms" food. Also, buying foods from markets may be associated with unhealthy diets such as higher fat, additives/preservatives, more sugar, chemical residues etc. Therefore, health impact studies comparing subsistence and market-oriented farm households are warranted. Lastly, considering the remote setting of villages, how can private retail food companies be best incentivized to ensure diverse foods accessible in rural areas, as against prevailing only periodical markets and grocery shops in the villages.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RN: conceptualization, methodology, validation, transcription, coding, formal analysis, investigation, data curation, and writing—original draft. SN: conceptualization, methodology, writing—review and editing, supervision, project administration, and funding acquisition. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Ambikapathi, R., Gunaratna, N. S., Madzorera, I., Passarelli, S., Canavan, C. R., Noor, R. A., et al. (2019). Market food diversity mitigates the effect of environment on 'women's dietary diversity in the Agriculture to Nutrition (ATONU) study, Ethiopia. *Public Health Nutr.* 22, 2110–2119. doi: 10.1017/S136898001900051X

Brannen, J., Brannen, J., and Brannen, J. (2005). *Mixed methods research: A discussion paper*. University of London, London, United Kingdom.

Census (2011). Census. Available online at: http://agcensus.dacnet.nic.in/MessagePage.htm?aspxerrorpath=/DL/districtT1table1.aspx (accessed March 6, 2019).

Chandra, Y., and Liang, E. S. (2016). *Qualitative Data Analysis With RQDA*. Singapore: Springer.

Creswell, J. W. (1999). "Mixed-method research," in *Handbook of Educational Policy*, ed G. J. Cizek (Amsterdam: Elsevier), 455–472. doi: 10.1016/B978-012174698-8/50045-X

Dillon, A., McGee, K., and Oseni, G. (2015). Agricultural production, dietary diversity and climate variability. *J. Dev. Stud.* 51, 976–995. doi: 10.1080/00220388.2015.1018902

Fanzo, J., Hunter, D., Borelli, T., and Mattei, F. (2013). *Diversifying Food and Diets: Using Agricultural Biodiversity to Improve Nutrition and Health*. London: Routledge. doi: 10.4324/9780203127261

Gupta, S., Sunder, N., and Pingali, P. L. (2020). Market access, production diversity, and diet diversity: evidence from India. *Food Nutr. Bullet.* 41, 167–185. doi: 10.1177/0379572120920061

Hirvonen, K., and Hoddinott, J. (2017). Agricultural production and 'children's diets: evidence from rural Ethiopia. *Agri. Econ.* 48, 469–480. doi: 10.1111/agec.12348

Hussein, F. M., Ahmed, A. Y., and Muhammed, O. S. (2018). Household food insecurity access scale and dietary diversity score as a proxy indicator of nutritional status among people living with HIV/AIDS, Bahir Dar, Ethiopia, 2017. *PLoS ONE* 13:e0199511. doi: 10.1371/journal.pone.0199511

Johnson, R. B., and Onwuegbuzie, A. J. (2004). Mixed methods research: a research paradigm whose time has come. *Educ. Res.* 33, 14–26. doi: 10.3102/0013189X033007014

Jones, A. D. (2016). On-farm crop species richness is associated with household diet diversity and quality in subsistence-and market-oriented farming households in Malawi. *J. Nutr.* 147, 86–96. doi: 10.3945/jn.116.235879

Jones, A. D., Shrinivas, A., and Bezner-Kerr, R. (2014). Farm production diversity is associated with greater household dietary diversity in Malawi: findings from nationally representative data. *Food Pol.* 46, 1–12. doi: 10.1016/j.foodpol.2014.02.001

Kashyap, P. (2016). *Rural Marketing, 3/e*. Chennai: Pearson Education India.

Kennedy, G., Ballard, T., and Dop, M. C. (2011). *Guidelines for Measuring Household and Individual Dietary Diversity*. Rome: Food and Agriculture Organization of the United Nations.

Kennedy, G., Berardo, A., Papavero, C., Horjus, P., Ballard, T., Dop, M., et al. (2010). Proxy measures of household food consumption for food security assessment and surveillance: comparison of the household dietary diversity and food consumption scores. *Public Health Nutr.* 13, 2010–2018. doi: 10.1017/S136898001000145X

Koppmair, S., Kassie, M., and Qaim, M. (2017). Farm production, market access and dietary diversity in Malawi. *Public Health Nutr.* 20, 325–335. doi: 10.1017/S1368980016002135

Kumar, D., Bassi, N., and Ganguly, A. (2016 August 20). Rejuvenating Tanks in Telangana. *Economic and Political Weekly*. p. 30–34.

Lenjiso, B. M., Smits, J. P. J. M., and Ruben, R. (2016). *Smallholder Milk Market Participation, Dietary Diversity and Nutritional Status Among Young Children in Ethiopia*, 147. Available online at: https://repository.ubn.ru.nl/handle/2066/157095 (accessed November 23, 2021).

Luckett, B. G., DeClerck, F. A., Fanzo, J., Mundorf, A. R., and Rose, D. (2015). Application of the nutrition functional diversity indicator to assess food system contributions to dietary diversity and sustainable diets of Malawian households. *Public Health Nutr*. 18, 2479–2487. doi: 10.1017/S136898001500169X

Ludwig, T. (2018). *An Egg for an Egg and a Bean for a Bean? How Production Diversity Determines Dietary Diversity of Smallholder Farmers in Rural India. How Production Diversity Determines Dietary Diversity of Smallholder Farmers in Rural India (January 4, 2018). ZEF-Discussion Papers on Development Policy*, 247. doi: 10.2139/ssrn.3098283

Morse, J. M. (2016). *Mixed Method Design: Principles and Procedures—Janice M Morse—Google Books*. London: Routledge. doi: 10.4324/9781315424538

Mulenga, B. P., Ngoma, H., and Nkonde, C. (2021). Produce to eat or sell: panel data structural equation modeling of market participation and food dietary diversity in Zambia. *Food Policy* 2021:102035. doi: 10.1016/j.foodpol.2021.102035

Muller, C. (2009). Do agricultural outputs of partly autarkic peasants affect their health and nutrition? Evidence from Rwanda. *Food Pol*. 34, 166–175. doi: 10.1016/j.foodpol.2008.10.010

Nandi, R., Nedumaran, S., and Ravula, P. (2021). The interplay between food market access and farm household dietary diversity in low and middle income countries: a systematic review of literature. *Glob. Food Secur*. 28:100484. doi: 10.1016/j.gfs.2020.100484

Pellegrini, L., and Tasciotti, L. (2014). Crop diversification, dietary diversity and agricultural income: empirical evidence from eight developing countries. *Can. J. Dev. Stud*. 35, 211–227. doi: 10.1080/02255189.2014.898580

Pinstrup-Andersen, P. (2007). Agricultural research and policy for better health and nutrition in developing countries: a food systems approach. *Agri. Econ*. 37, 187–198. doi: 10.1111/j.1574-0862.2007.00244.x

Powell, B., Thilsted, S. H., Ickowitz, A., Termote, C., Sunderland, T., and Herforth, A. (2015). Improving diets with wild and cultivated biodiversity from across the landscape. *Food Secur*. 7, 535–554. doi: 10.1007/s12571-015-0466-5

Qaim, M., Kibrom, T., and Krishna, V. V. (2016). Market access and farm household dietary diversity. *Rural*. 21, 12–14. Available online at: https://www.rural21.com/fileadmin/downloads/2016/en-01/rural2016_01-S12-14.pdf

Satyam, A. R. (2018), "Reflections from a periodic market in rural india," in *Bottom of the Pyramid Marketing: Making, Shaping and Developing BoP Markets*, ed R. Singh (Bingley: Emerald Publishing Limited), 135–147.

Sekabira, H., and Nalunga, S. (2020). Farm production diversity: is it important for dietary diversity? Panel data evidence from Uganda. *Sustainability* 12:31028. doi: 10.3390/su12031028

Sharma, P. K. (2017). Pattern of crop concentration and crop diversification-an economic analysis. Maharashtra. *J. Agril. Econom*. 20, 128–132.

Sibhatu, K. T., Krishna, V. V., and Qaim, M. (2015). Production diversity and dietary diversity in smallholder farm households. *Proc. Natl. Acad. Sci. U. S. A*. 112, 10657–10662. doi: 10.1073/pnas.1510982112

Sibhatu, K. T., and Qaim, M. (2017). Rural food security, subsistence agriculture, and seasonality. *PLoS ONE* 12:e0186406. doi: 10.1371/journal.pone.0186406

Sibhatu, K. T., and Qaim, M. (2018). Review: meta-analysis of the association between production diversity, diets, and nutrition in smallholder farm households. *Food Policy* 77, 1–18. doi: 10.1016/j.foodpol.2018.04.013

Snapp, S. S., and Fisher, M. (2015). "Filling the maize basket" supports crop diversity and quality of household diet in Malawi. *Food Secur*. 7, 83–96. doi: 10.1007/s12571-014-0410-0

Swindale, A., and Bilinsky, P. (2006). *Household Dietary Diversity Score (HDDS) for Measurement of Household Food Access: Indicator Guide (Version 2)*.

Vanessa Cordero-Ahiman, O., Santellano-Estrada, E., and Garrido, A. (2017). Dietary diversity in rural households: the case of indigenous communities in Sierra Tarahumara, Mexico. *J. Food Nutr. Res*. 5, 86–94. doi: 10.12691/jfnr-5-2-3

Velayudhan, S. K. (2016). *Relevance of Rural Periodic Markets: A Review (No. 199; Working Papers)*. Indian Institute of Management Kozhikode. Available online at: https://ideas.repec.org/p/iik/wpaper/199.html (accessed November 23, 2021).

Verger, E. O., Ballard, T. J., Dop, M. C., and Martin-Prevel, Y. (2019). Systematic review of use and interpretation of dietary diversity indicators in nutrition-sensitive agriculture literature. *Glob. Food Secur*. 20, 156–169. doi: 10.1016/j.gfs.2019.02.004

Webb, P. (2014). *Nutrition and the Post-2015 Sustainable Development Goals: A Technical Note*. United Nations Systems, Bonn, Germany.

Wiesmann, D., Bassett, L., Benson, T., and Hoddinott, J. (2009). *Validation of the World Food 'Programme's Food Consumption Score and Alternative Indicators of Household Food Security*. Washington, DC: International Food Policy Research Institute (No. 00870).

World Food Program. (2008). *Technical Guidance Sheet. Food Consumption Analysis. Calculation and Use of the Food Consumption Score in Food Consumption and Food Security Analysis*. Rome: FAO.

Zanello, G., Shankar, B., and Poole, N. (2019). Buy or make? Agricultural production diversity, markets and dietary diversity in Afghanistan. *Food Policy* 87:101731. doi: 10.1016/j.foodpol.2019.101731

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Evaluating the Effects of Wheat Cultivar and Extrusion Processing on Nutritional, Health-Promoting, and Antioxidant Properties of Flour

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Wheat has been considered one of the most important staple foods for thousands of years. It is one of the largest suppliers of calories in the daily diet, which is added to many different products. Wheat is also a good source of health-benefiting antioxidants. This study aims toinvestigate the changes in the antioxidant properties, such as total phenol content, 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating activity, 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$) scavenging activity, and color intensity, during the extrusion processing of six different wheat cultivars. The extrusion factors evaluated were 15% feed moisture and two extrusion temperatures (150 and 180°C). Extrusion processing increased antioxidant activity (DPPH, metal chelating activity, and $ABTS^+$ scavenging activity), whereas total flavonoids content and total phenolic content were decreased. The L* values of wheat flours increased significantly ($p < 0.05$) after extrusion at 150 and 180°C, 15% mc. Furthermore, redness was decreased from control wheat cultivars (range: 0.17–0.21) to extrusion at 150°C (range: 0.14–0.17) and 180°C (range: 0.1–0.14). The study suggests that extruded wheat could improve the antioxidant potential in food products.

Keywords: wheat, extrusion, total phenolics, antioxidants, redness intensity

INTRODUCTION

Processing methods of foods can modulate the polyphenol content of foods in several ways (1). Extrusion of cereal-based products has advantages over other usual processing methods because of low cost, short time, high productivity, versatility, unique product shapes, and energy savings (2). It is a versatile and efficient technology involving high temperature, pressure, and short time among different processing methods. It is used to develop infant foods, snack foods, ready-to-eat breakfast cereals, and pet foods, among other products. Extrusion technology is a new economical processing method; it can achieve protein, starch, and cellulose polymer transformation directly or indirectly in a short time (3–5).

Antioxidants as health-promoting food components have been discussed in nutrition science for several years (6–8). The natural or enriched content of antioxidants is associated with health and disease prevention (9, 10). Phenolic acids are the main antioxidants in cereal grains that seem to have enough potential to be beneficial to health by scavenging free radicals, inhibiting lipid peroxidation, and thus exhibiting anti-cancer activity (11, 12). Free radicals are highly reactive forms that persist during the routine metabolic process in our body (13). They are responsible for cell damage which may ultimately lead to diseases. To counteract the negative effect of free radicals, one should eat an antioxidant-rich diet in a routine dietary chart (14). Wheat has been one of the most important staple foods for thousands of years. It is rich in basic nutrients a source of energy, protein, vitamins, and minerals, and contains important amounts of dietary fibers along with bioactive compounds and antioxidant properties (15). Despite the well-known health benefits, recommendations, labeling, and communication campaigns, the majority of cereal foods are made from refined wheat flour and contain less dietary fiber and other health-promoting compounds when compared to whole grain raw materials. Because of its high gluten content, wheat flour is very suitable for making bread and other bakery products. The use of whole grains wheat flour in extruded products can be an effective alternative for obtaining healthier breakfast cereals. The objective of the present study was to provide a comprehensive summary of the changes in the antioxidant properties, such as total phenol content, DPPH, metal chelating activity, $ABTS^+$ scavenging activity, and color intensity during the extrusion processing of different wheat cultivars. Diversity is worthwhile because every wheat variety has something to offer in terms of ingredients, taste, and possible uses.

MATERIALS AND METHODS

Wheat Varieties

For this study, six wheat cultivars (cv) (wheat varieties *viz.* PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) were collected from Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, Haryana, India. The grain of each variety was cleaned and stored (5°C) for further evaluation. All tests were performed in triplicates on a dry weight basis.

De-husking, Milling, and Extrusion of Wheat

Dehusking of wheat samples was carried out using a rice miller, as reported by Punia and Sandhu (16). In the polishing chamber, hulled wheat (150 g) was transferred, and the polisher was run until the husk was fully removed from the grain. The whole wheat flour was made by grinding de-husked wheat in a Super Mill (Newport Scientific, Australia), conditioning it to 15% feed moisture content (mc), and storing it in polyethylene bags for 12 h. A corotating twin-screw extruder was used for the extrusion (Clextral, BC 21, Firminy, France). The screw diameter, (L/D) ratio and die diameter were adjusted to 25 mm, 16 mm, and 6 mm, respectively. The screw speed (400 rpm) and feed rate (20 kg/h) were kept constant. Wheat flour was extruded at 150 and 180°C with barrel temperatures of 50, 100, 125, and 150°C (for 150°C) and 50, 100, 140, and 180°C (for 180°C). An induction heating belt warmed the terminal section of the barrel while running water cooled the feeding section.

Antioxidant Activity

DPPH Radical Scavenging Activity Assay

A modified version of the method described by Brand-Williams et al. (17) was used to measure 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Methanol-containing solution of the free radical DPPH has been used. In brief, 100 mg of ground wheat samples were extracted for 2 h in 1 ml methanol and centrifuged for 10 min at 3,000 rpm. The supernatant (100 μl) was treated with 3.9 ml of a DPPH solution containing 6 x 10^{-5} mol/l. Absorbance (A) at 517 nm was recorded after the solution was stored in a dark place for 30 min at 25°C.

The DPPH activity was calculated using the following formula (18, 19):

$$DPPH\ (\%) = \frac{Abs_{DPPH} - Abs_{sol}}{Abs_{DPPH}} \times 100$$

where $Abs_{DPPH} =$ Absorbance of DPPH solution and $Abs_{sol} =$ Absorbance of extracts.

Metal Chelating Fe^{2+} Activity

The already published method described by Dinis et al. (20) was used to determine metal chelating activity. Briefly, 50 μl of ferrous chloride (2 mM/l) was mixed with 0.5 ml extract, and 1.6 ml of 80% methanol was added. After 5 min, the reaction was started by adding 5 mM/l ferrozines (100 μl) and vortex shaking the mixture. For 10 min, the mixture was incubated at room temperature (25°C). A spectrophotometer (UV-VIS-NIR Spectrophotometer UV-3600 Plus Series, Shimadzu, Kyoto, Japan) was used to measure the absorbance (A) of the solution at 562 nm. The following formulae were used to calculate the extract's Fe^{2+} chelating activity (7):

$$\text{Iron } (Fe^{2+}) \text{ chelating activity } \% = \{1 - (\text{A of sample at 562nm/Absorbance of control at 562nm})\} \text{x } 100$$

$ABTS^+$ Scavenging Activity

Generally known as the ABTS, the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma–Aldrich Chemie, Steinheim, Germany) is a spectrophotometric method for determining the antioxidant properties of different compounds. An improved ABTS decolorization test was used in the study (21). The oxidation of ABTS using potassium persulfate (BDH, Poole, UK) generates $ABTS^+$. In a disposable microcuvette, 3 ml of ABTS cation solution was combined with 30 μl extract, and the decrease in absorbance was evaluated after 1 min of incubation. Different concentrations of vitamin C were used to create a standard curve. The scavenging ability of $ABTS^+$ was measured in μmol of ascorbic acid equivalents (VEAC) per gram of wheat.

Total Phenolic Content

Total phenolic content (TPC) in studied extracts was evaluated using the already published method described by Gao et al. (22) using the Folin-Ciocalteu- Sigma-Aldrich, Buenos Aires, Argentina reagent. Briefly, 200 mg (wheat flour) were extracted for 2 h at room temperature (25°C) with 4 ml acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) using a wrist action shaker. On a centrifuge, the mixture was centrifuged at 3,000 g for 10 min (REMI, Mumbai, India). The total phenolic content of the supernatant was determined. A 200 μl aliquot of the extract was added to a 1.5 ml Folin–Ciocalteu reagent that had been freshly diluted (10-fold). After allowing the liquid to equilibrate for 5 min, 1.5 ml of sodium carbonate solution (60 g/l) was added. The absorbance of the combination was measured at 725 nm after 90 min of incubation at room temperature (25°C). As a blank, acidified methanol was used. The results were calculated in gallic acid equivalents (GAE)/g of flour. All of the analyses were carried out in duplicate.

Total Flavonoid Content

Jia et al. (23) previously described the method for determining total flavonoids content, and for this study same method has been used. In brief, 1.25 ml of distilled water was used to dilute the wheat extract (250 μl). The sodium nitrite (75 μl of a 5% solution) was added, and the mixture was allowed to stand for 6 min. In addition, 150 μl of a 10% aluminum chloride solution was added, and the combination was allowed to react for 5 min. Thereafter, the solution was then stirred well with 0.5 ml of 1M sodium hydroxide. A Spectrophotometer-Thermo-scientific was used to detect the absorbance at 510 nm. The standard was catechin, and the results were expressed as g of catechin equivalents (CE)/g of flour.

Color Characteristics

Color measurement of flours has been analyzed based on the L*, a*, b* color scheme, and a Hunter Colorimeter with an optical sensor (Hunter Associates Laboratory Inc. Restan VA., USA) was used. The color difference (ΔE) was calculated as:

$$\Delta E = \{(dL^*) + (da^*) + (db^*)\}^{1/2}$$

Redness intensity (RI = a*/b*) was calculated for each sample cultivar (PBW-343, WH-896, WH-1080, PBW-590, WH-283, WHD-943).

DATA ANALYSIS

All of the tables' data is an average of triplicate observations that were subjected to one-way ANOVA, applying Minitab statistical software version 14 (Minitab Inc., USA).

RESULT AND DISCUSSION

Antioxidant Activity

DPPH Radical Scavenging Activity Assay

The DPPH procedure assesses the target compounds' hydrogen donating capacity in a methanolic media. Antioxidative activities of wheat flour samples with extrusion are shown in **Figure 1**. Percent DPPH inhibition shown by control (non-extruded) wheat cultivars ranged from 13.2 to 21.6%, with cv.WH-283 and cv.PBW-343 shows the highest and the lowest values, respectively. Compared to control wheat samples, extrusion cooking significantly increased DPPH free radical scavenging activity in all wheat cultivars. Antioxidant activity varied significantly among cultivars when the extrusion was carried out at 180°C and 15% mc. The DPPH ranged from 16.7 to 22.6%, with the highest and lowest being cv.WHD-943 and cv. PBW-343, respectively. Also, the DPPH in the extrudates (150°C, 15% mc) varied significantly among cultivars that ranged between 19.8 and 33.5%. The highest and the lowest increase were exhibited by cv. WHD-943 and cv. WH-1080, respectively. Rufian-Henares and Delgado-Andrade (24) and Punia Bangar et al. (7) reported an increase in the antioxidant activity of rye and barley, which could be related to the development of Maillard browning pigments, which boosted the antioxidant activity of extrudates (25, 26).

Metal Chelating Fe^{2+} Activity

Extrusion cooking considerably boosted metal-chelating Fe^{2+}activity in all cultivars studied as compared to their corresponding control samples. Metal chelating activity varied substantially ($p < 0.05$) among non-extruded wheat cultivars, ranging from 22 to 42% (**Figure 2**), with cv.WH-283 and cv.WH-896 having the highest and lowest levels, respectively. When extrusion was carried out at 180°C, and the feed moisture was 15%, the metal chelating activity varied significantly among the cultivars and ranged between 26.6 and 43.4%. The highest and the lowest metal chelating activity was exhibited by cv.WH-283 (43.4%) and cv.PBW-343 (26.6%). When the feed moisture was sustained at 15%, and temperature at 150°C, the metal chelating activity in all wheat cultivars increased and ranged from 31.3 to 54.4%. Cv. WH-283 exhibited the highest, while cv. WH-896 showed the lowest metal chelating power. An increase in the metal chelating activity may be due to the formation of melanoidins during thermal processing (27). Rufian-Henares and Delgado-Andrade (24) reported that the increase in antioxidant activity could be explained by the formation of Maillard browning pigments, which enhanced the antioxidant activity of extruded products.

$ABTS^+$ Activity

The antioxidative capacity of test compounds is assessed by measuring their ability to reduce the ABTS radical anion to its non-radical form. In control wheat cultivars, ABTS scavenging activity ranged from 3.06 to 8.11 μmole VEAC/g, the highest was observed for cv. WH-283 and cv. PBW-343 showed the lowest. Compared to non-extruded samples, extrusion cooking resulted in a considerable increase in ABTS free radical scavenging activity in all cultivars (**Figure 3**). The ABTS radical scavenging activity in 180^0C and 15% mc extrudates varied insignificantly ($p < 0.05$) among wheat cultivars and ranged from 5.44 to 12.6 μmol/g, with the highest and lowest being for cv. WH-283 and cv. PBW-343. Further, the ABTS radical scavenging activity in 180°C and 15% mc wheat varieties (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) extrudates varied significantly ($p <$

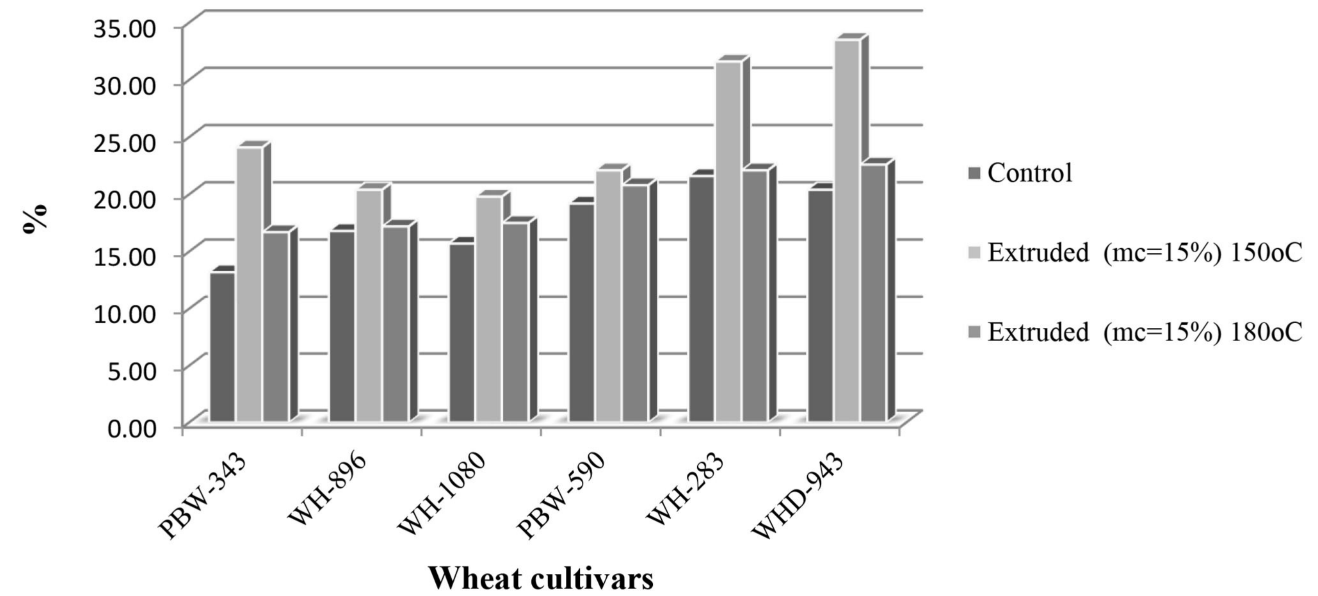

FIGURE 1 | 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay (%) of control and extruded wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) flours at different temperatures (150 and 180°C, mc = 15%).

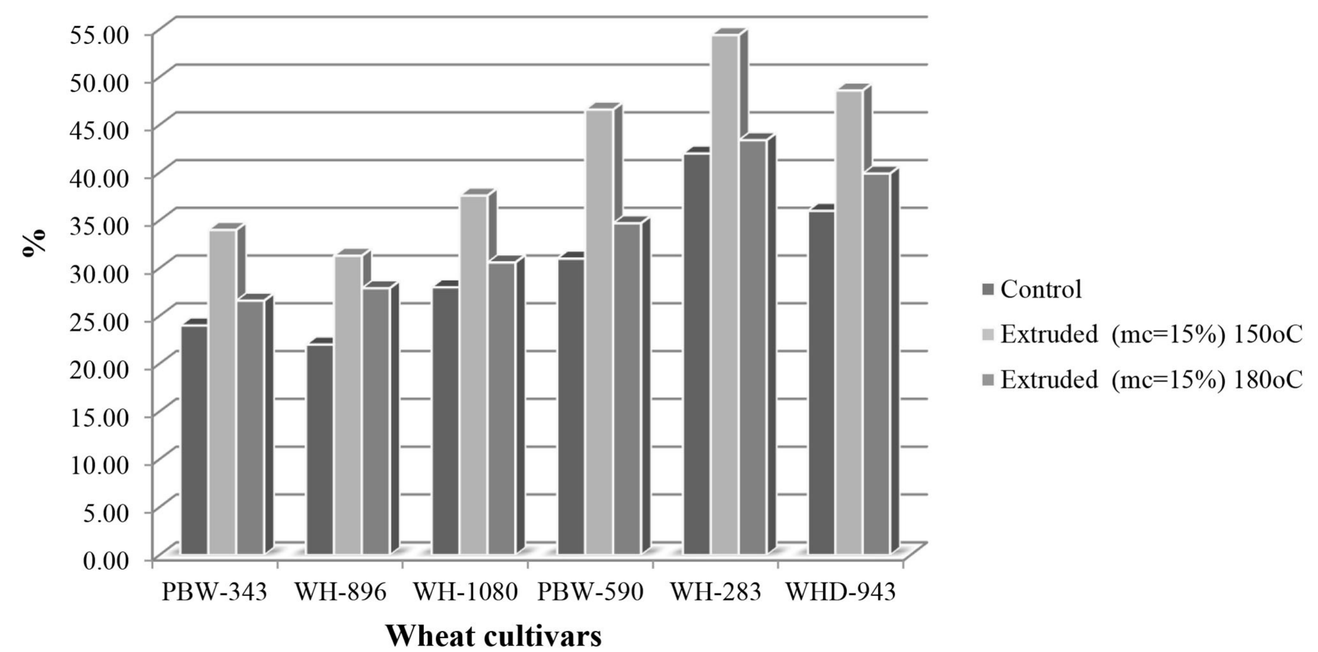

FIGURE 2 | Metal chelating iron (Fe^{2+}) activity(%) of control and extruded wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) flours at different temperature (150 and 180°C, mc = 15%).

0.05) among cultivars and ranged from 8.23 to 20.3 µmol/g. The highest and the lowest were observed for cv. WHD-943 and cv. PBW-343, respectively. According to studies (24, 28), pigments (especially melanoidins) are widely known to have antioxidant action. The development of Maillard browning pigments, which boosted the antioxidant activity of extruded goods, could explain the increased antioxidant activity.

Total Phenolic Content

The total phenolic content (TPC) in the wheat cultivars ranged from 974 to 1,399 µg GAE/g (**Figure 4**). Vaher et al. (29) reported TPC of winter and spring wheat varieties ranged from 892 to 569 µg/g. Changes in the TPC can be attributed to differences in wheat cultivars and extraction solvent. When compared to control (non-extruded) wheat samples, the total phenolic content

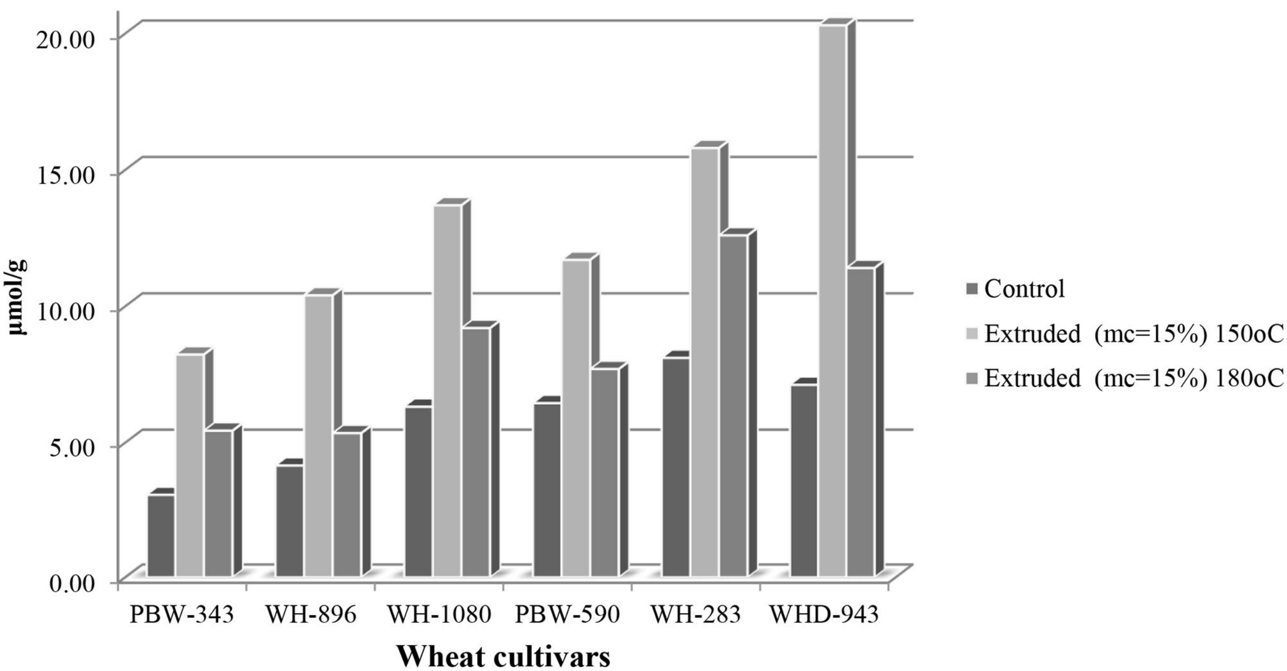

FIGURE 3 | 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$) activity (µmol/g) of control and extruded wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) flours at different temperatures (150 and 180°C, mc = 15%).

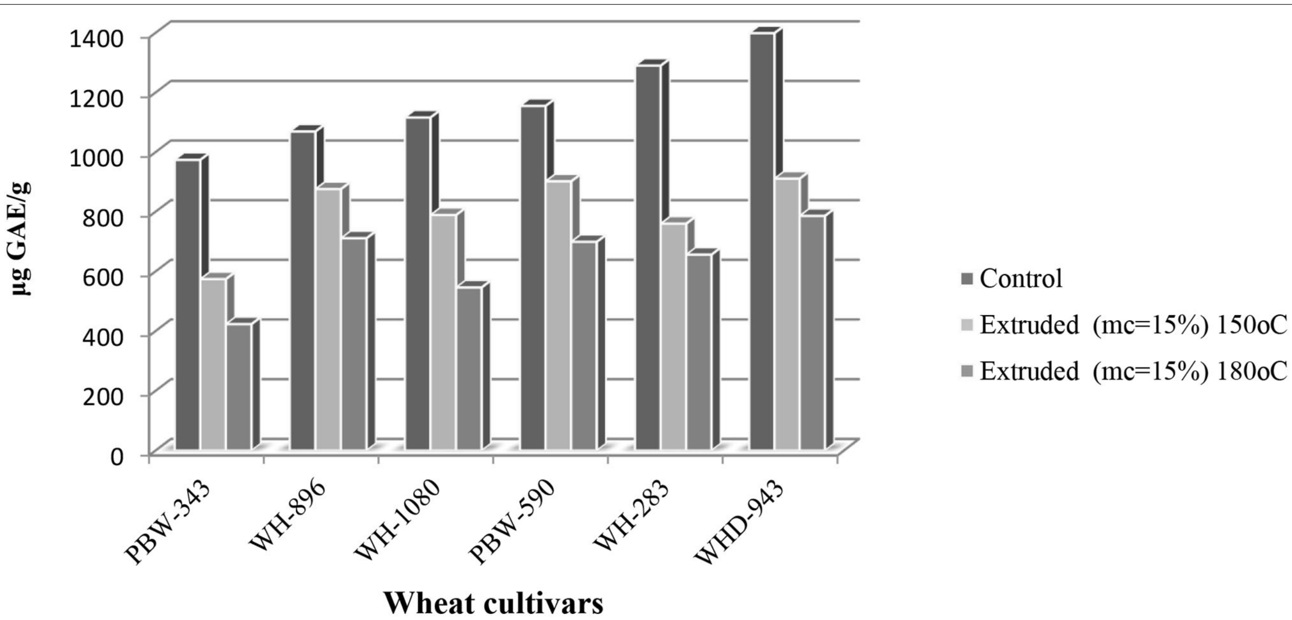

FIGURE 4 | Total phenolic content [TPC (µg GAE /g)] of control and extruded wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) flours at different temperatures (150 and 180°C, mc = 15%).

of all cultivars decreased considerably after extrusion. TPC at 180°C and 15% moisture, varied significantly ($p < 0.05$) among cultivars and ranged between 423 to 786 µg GAE/g. The highest and the lowest were observed for cv.WHD-943 and cv.PBW-343. At 150°C and 15%, mc TPC varied significantly ($p < 0.05$) among cultivars and ranged from 524 to 911 µg GAE/g, the highest and the lowest being for cv.WHD-943 and cv.PBW-343, respectively. Sharma et al. (30) reported a decrease in TPC in HTLM (180°C, 15% mc) and LTLM (150°C, 15% mc) extrudates of wheat cultivars. Korus et al. (31) investigated the effect of extrusion on polyphenol content and antioxidant activity of common bean. It has been observed a significant

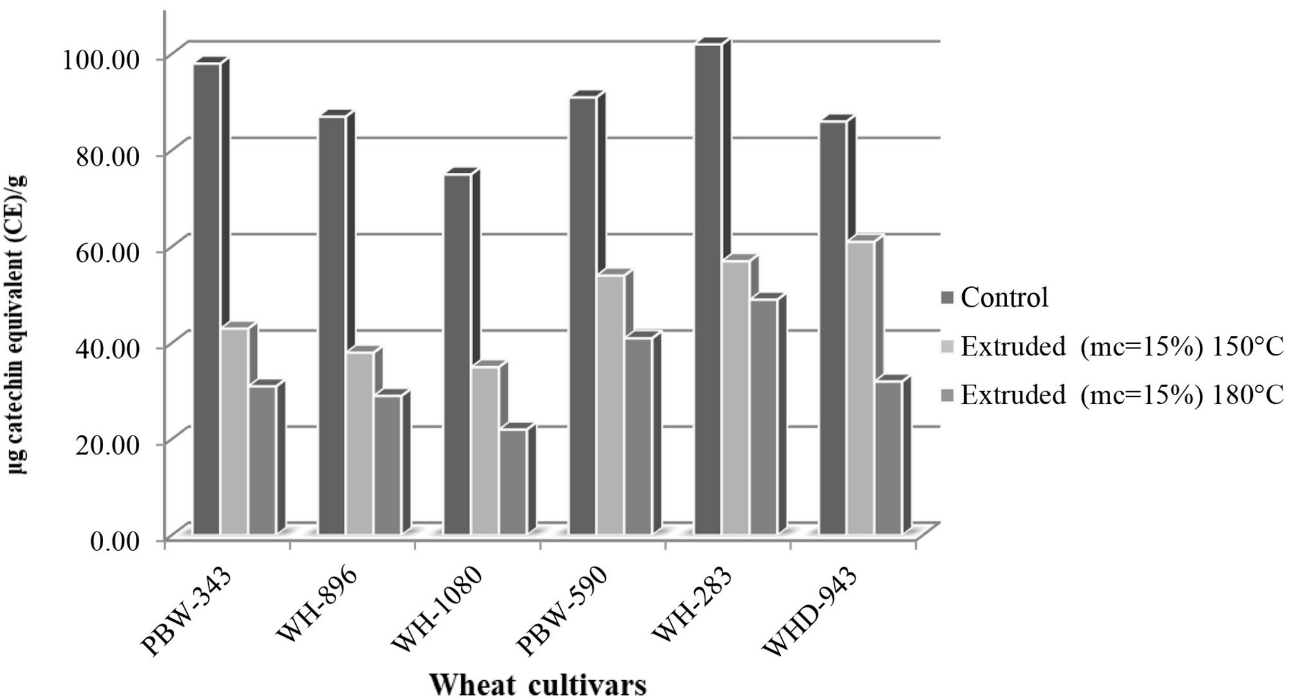

FIGURE 5 | Total flavonoid content [TFC (µg catechin equivalent (CE)/g)] of control and extruded wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) flours at different temperature (150 and 180°C, mc = 15%).

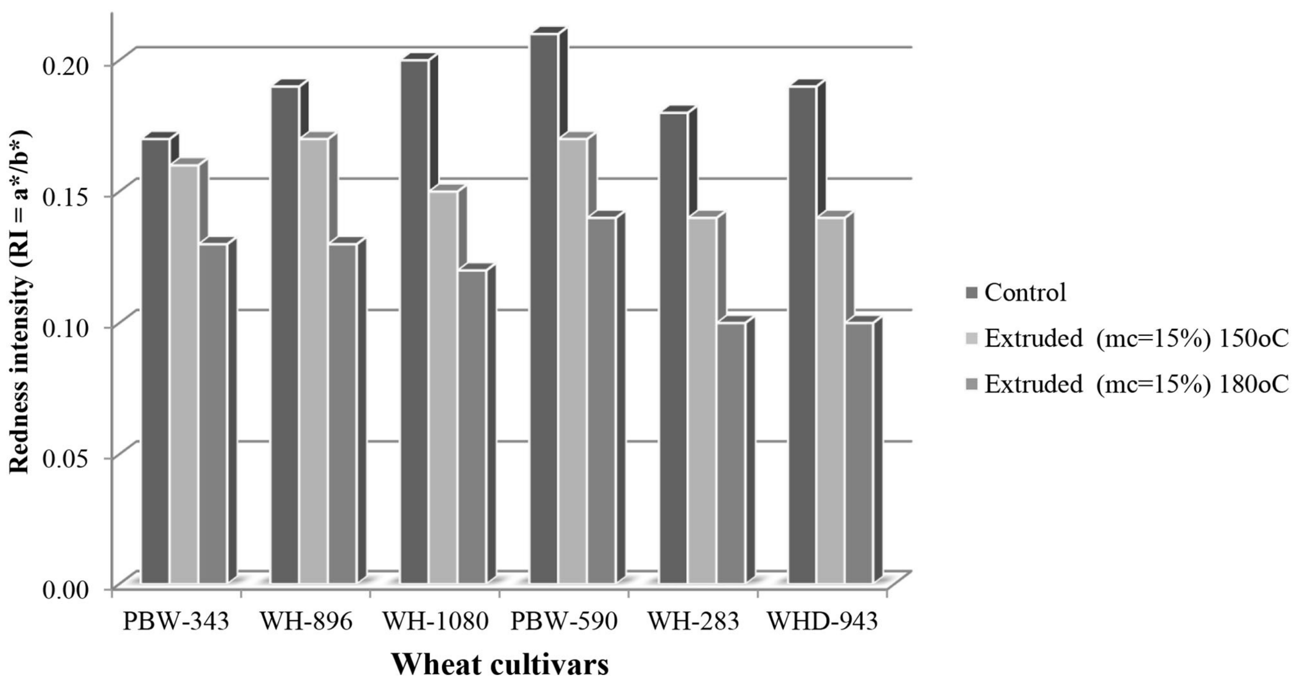

FIGURE 6 | Graphic representation of redness intensity (RI = a*/b*) between control and wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) at different temperatures (150°C and 180°C, mc = 15%).

decrease in polyphenol content and antioxidant activity. Repo-Carrasco-Valencia et al. (32) reported that phenolic compounds during extrusion might undergo decarboxylation due to high barrel temperature, and high moisture content may promote polymerization of phenols leading to reduced extractability and antioxidant activity on one side. Thermal treatment induced

TABLE 1 | Color parameters of extruded and non-extruded wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, and WHD-943) at different temperatures (150 and 180°C, mc = 15%).

Wheat cultivars	Samples	L	a*	b*	RI = a*/b*
PBW 343	Control	71.2	1.62	9.31	0.17
	Extruded 150°C	74.3	1.51	9.11	0.16
	Extruded 180°C	79.4	1.34	10.22	0.13
WH-896	Control	74.4	2.57	13.2	0.19
	Extruded 150°C	76.9	2.41	14.3	0.17
	Extruded 180°C	82.6	2.02	15.1	0.13
WH-1080	Control	75.2	2.08	10.4	0.20
	Extruded 150°C	78	1.91	12.8	0.15
	Extruded 180°C	81.3	1.83	14.6	0.12
PBW-590	Control	78.8	2.26	10.6	0.21
	Extruded 150°C	82.8	2.12	12.3	0.17
	Extruded 180°C	85.3	1.98	13.5	0.14
WH-283	Control	55.9	1.55	8.49	0.18
	Extruded 150°C	59.8	1.31	9.3	0.14
	Extruded 180°C	61.4	1.20	11.2	0.10
WHD-943	Control	75.8	2.62	13.3	0.19
	Extruded 150°C	77.8	2.03	14.4	0.14
	Extruded 180°C	79.6	1.82	16.9	0.10

*As shown in this Table and **Figure 6,** RI decreased from control (range: 0.17–0.21) to 150°C (range: 0.14–0.17) to 180°C (range: 0.10–0.14), and differences between wheat cultivars (PBW-343, WH-896, WH-1080, PBW-590, WH-283, WHD-943) are representative.*

the hydrolysis of conjugated phenolic compounds resulting in the release of free phenolic acids (33). On the other side, according to Ramos-Enríquez et al. (32), the extrusion process does not cause an increase in the release of TPC due to the addition of methanol during the extraction of TPC. Besides, feed moisture and temperature significantly affect TPC, as already reported (34).

Total Flavonoid Content

Flavonoids' antioxidant mechanism may be due to interactions between flavonoids and metal ions, particularly iron and copper (35). The total flavonoid content (TFC) of control wheat ranged from 75 to 102 μg CE/g and varied significantly ($p < 0.05$) among control wheat cultivars (**Figure 5**), cv. WH-283 had the highest content and cv. WH-1080 has the lowest content. A significant decrease in the TFC was observed during extrusion cooking. TFC levels may have decreased due to the thermal destruction of heat-sensitive flavonoids (36). TFC varied significantly among cultivars in the extrudates (180°C and 15% mc) and ranged from 22 to 49 μg catechin equivalent (CE)/g. TFC varied significantly among wheat cultivars in extrudates (150°C and 15% mc) and ranged from 35 to 61 μg catechin equivalent (CE)/g. as shown in **Figure 1**. It has also been shown that flavonoids and phenols interact with proteins, causing a loss of their effectiveness (37). Zhu et al. (38) reported that flavonoids are heat susceptible phenolic compounds; therefore, heat exposure during roasting could be a reason for the decrease in TFC.

Color Characteristics

Hunter Colorimeter was used to analyze the ground wheat extrudates. The lightness is indicated by the L* value, which ranges from 0 to 100. When compared to their equivalent control samples, the lightness of extrudates dropped dramatically. Color characteristics are shown in **Table 1**. The L* of extrudates at 180^0C varied significantly (**Table 1**), and a significant increase in lightness was observed. Cv. PBW-590 and cv. WH-283 exhibited the highest and lowest value of L* (85.3 and 61.4) after extrusion. Extrudates at 150^0C also showed a significant variation in L* among the cultivars that ranged from 59.8 to 82.8. The moisture content of feed material (wheat flour and brewer's spent grain) had a substantial effect on the L values, also according to Stojceska et al. (39, 40). Extrusion cooking of barley and their blends with tomato pomace resulted in a decrease in lightness, according to Altan et al. (41). The redness (a*) varied significantly within the cultivars and ranged from 1.55 to 2.57, and after extrusion, the redness significantly decreased both at 180°C and 150°C. The yellowness (b*) of wheat flours from control cultivars varied significantly within the cultivars and ranged from 8.49 to 13.3, and upon extrusion, at 150°C, the b value decreased and ranged between 9.3 and 14.4 and at 180°C the b value increases, the highest for cv. WHD-943 and the lowest for cv. WH-283 was observed.

CONCLUSION

During the present investigation, the effect of extrusion on the antioxidant properties, such as total phenol content, DPPH, metal chelating activity, $ABTS^+$ scavenging activity, and color intensity of different wheat cultivars (wheat varieties WH-283, WHD-943, PBW-590, WH-1080, WH-896, and PBW-343), was studied. Results demonstrated that the feed moisture (15%) and extrusion at different temperatures (150 and 180°C) could improve the wheat flour quality. Extrusion increased antioxidant activity (DPPH, metal chelating activity, and $ABTS^+$ scavenging activity), compared with total flavonoids content and total phenolic content, which were decreased. Antioxidant-rich flour could be utilized at a commercial scale for the preparation of health-benefiting food/bakery products. An elaborated study could be designed to optimize the processing conditions in this context. Diversity is worthwhile because every wheat variety has something to offer in terms of ingredients, taste, possible uses, and further food and feed applications.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

All authors participated in the performing, generating, and interpretation of results.

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REFERENCES

1. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr.* (2004) 79:727–47. doi: 10.1093/ajcn/79.5.727
2. Annica AM, Andersson R, Andersson AJ, Andersson J, Fredriksson H. Effect of different extrusion parameters on dietary fiber in wheat bran and rye bran. *J Food Sci.* (2017) 82:1344–50. doi: 10.1111/1750-3841.13741
3. Valentina S, Paul A, Andrew P, Senol L. The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chem.* (2010) 121:156–64. doi: 10.1016/j.foodchem.2009.12.024
4. Alam MS, Kaur J, Khaira H, Gupta K. Extrusion and extruded products: changes in quality attributes as affected by extrusion process parameters: a review. *Crit Rev Food Sci Nutr.* (2016) 56:445–75. doi: 10.1080/10408398.2013.779568
5. Punia Bangar S, Purewal SS, Trif M, Maqsood S, Kumar M, Manjunatha V, et al. Functionality and applicability of starch-based films: an eco-friendly approach. *Foods.* (2021) 10:2181. doi: 10.3390/foods10092181
6. Purewal SS, Kamboj R, Sandhu KS, Kaur P, Sharma K, Kaur M, et al. Unraveling the effect of storage duration on antioxidant properties, physicochemical and sensorial parameters of ready to serve kinnow-amla beverages. *Appl Food Res.* (2022) 2. doi: 10.1016/j.afres.2022.100057
7. Punia Bangar S, Singh Sandhu K, Trif M, Rusu A, Pop ID, Kumar M. Enrichment in different health components of barley flour using twin-screw extrusion technology to support nutritionally balanced diets. *Front Nutr.* (2022) 8:823148. doi: 10.3389/fnut.2021.823148
8. Farooq M, Azadfar E, Trif M, Jabaleh RA, Rusu A, Bahrami Z, et al. Soybean oil enriched with antioxidants extracted from watermelon (*Citrullus colocynthis*) skin sap and coated in hydrogel beads via ionotropic gelation. *Coatings.* (2021) 11:1370. doi: 10.3390/coatings111 11370
9. Rusu AV, Criste FL, Mierliță D, Socol CT, Trif M. Formulation of lipoprotein microencapsulated beadlets by ionic complexes in algae-based carbohydrates. *Coatings.* (2020) 10:302. doi: 10.3390/coatings10030302
10. Polat S, Trif M, Rusu A, Šimat V, Cagalj M, Alak G, et al. Recent advances in industrial applications of seaweeds. *Crit Rev Food Sci Nutr.* (2021) 8:1–30. doi: 10.1080/10408398.2021.2010646
11. Baublis AJ, Cyldesdale FM, Decker EA. Whole grains and health: an overview. *J Am Coll Nutr.* (2000) 19(3 Suppl):289S—90S. doi: 10.1080/07315724.2000.10718962
12. Jurj A, Pop LA, Zanoaga O, Ciocan-Cârtită CA, Cojocneanu R, Moldovan C, et al. New Insights in gene expression alteration as effect of paclitaxel drug resistance in triple negative breast cancer cells. *Cell Physiol Biochem.* (2020) 54:648-64. doi: 10.33594/000000246
13. Braicu C, Gulei D, Raduly L, Harangus A, Rusu A, Berindan-Neagoe I. Altered expression of miR-181 affects cell fate and targets drug resistance-related mechanisms. *Mol Aspects Med.* (2019) 70:90–105. doi: 10.1016/j.mam.2019.10.007
14. Rusu AV, Alvarez Penedo B, Schwarze A, Trif M. The influence of candida spp in intestinal microbiota diet therapy, the emerging conditions related to candida in athletes and elderly people. In: Amornyotin S, editor. *Update in Geriatrics*. IntechOpen (2020). doi: 10.5772/intechopen.92791
15. Călinoiu LF, Vodnar DC. Whole grains and phenolic acids: a review on bioactivity, functionality, health benefits and bioavailability. *Nutrients.* (2018) 10:1615. doi: 10.3390/nu10111615
16. Punia S, Sandhu KS. Functional and antioxidant properties of different milling fractions of indian barley cultivars. *Carpathian J Food Sci Technol.* (2015) 7:19–27.
17. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate the antioxidant activity. *LWT - Food Sci Technol.* (1995) 28:25–30.
18. Trif M, Socaciu C. Evaluation of effiency, release and oxidation stability of seabuckthorn microencapsulated oil using Fourier transformed infrared spectroscopy. *Chem Listy.* (2008) 102:s1198–9.
19. Farooq M, Azadfar E, Rusu A, Trif M, Poushi MK, Wang Y. improving the shelf life of peeled fresh almond kernels by edible coating with mastic gum. *Coatings.* (2021) 11:618. doi: 10.3390/coatings11060618
20. Dinis TCP, Madeira VMC, Almeidam LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxylradicals scavengers. *Arch Biochem Biophys.* (1994) 315:161–9.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* (1999) 26:1231–7.
22. Gao L, Wang S, Oomah BD, Mazza G. Wheat quality: antioxidant activity of wheat millstreams. In: Ng P, Wrigley W, editors. *Wheat Quality Elucidation*. St. Paul, MN: American Association of Cereal Chemists (2002). p. 219–33.
23. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* (1998) 64:555–9.
24. Rufián-Henares JA, Delgado-Andrade C. Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. *Food Res Int.* (2009) 42:394e400. doi: 10.1016/j.foodres.2009.01.011
25. Zielinski H, Troszynska A. Antioxidant capacity of raw and hydrothermal processed cereal grains. *Polish J Food Nutr Sci.* (2000) 9:79–83.
26. Punia Bangar S, Trif M, Özogul F, Kumar M, Chaudhary V, Vukic M, et al. Recent developments in cold plasma-based enzyme activity (browning, cell wall degradation, and antioxidant) in fruits and vegetables.

Comp Rev Food Sci Food Safe. (2022) 21:1958–78. doi: 10.1111/1541-4337.12895

27. Shih MC, Kuo CC, Chiang W. Effects of drying and extrusion on colour, chemical composition, antioxidant activities and mitogenic response of spleenlymphocytes of sweet potatoes. *Food Chem.* (2009) 117:114–21. doi: 10.1016/j.foodchem.2009.03.084
28. Manzocco L, Calligaris S, Masrrocola D, Nicoli MC, Lerici CR. Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends Food Sci Tech.* (2000) 11: 340–6. doi: 10.1016/S0924-2244(01)00014-0
29. Vaher M, Masto K, Levandi T, Helmja K, Kaljurand M. Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chem.* (2010) 2:76–82. doi: 10.1016/j.proche.2009.12.013
30. Sharma P, Gujral HS, Singh B. Antioxidant activity of barley as affected by extrusion cooking. *Food Chemistry.* (2012) 131:1406–13.
31. Korus J, Gumul D, Czechowska K. Effect of extrusion on the phenolic composition and antioxidant activity of dry beans of phaseolus vulgaris l. *Food Technol Biotechnol.* (2007) 45:139–46.
32. Repo-Carrasco-Valencia R, de La Cruz AA, Alvarez JCI, Kallio H. Chemical and functional characterization of kaiwa (*Chenopodium pallidicaule*) grain, extrudate and bran. *Plant Foods Hum Nutr.* (2009) 64:94–101. doi: 10.1007/s11130-009-0109-0
33. Galvez Ranilla L, Genovese MI, Lajolo F. Effect of different cooking conditions on phenolic compounds and antioxidant capacity of some selected Brazilian bean (*Phaseolus vulgaris L.*) cultivars. *J Agricult Food Chem.* (2009) 57:5734–42. doi: 10.1021/jf900527v
34. Ramos-Enríquez JR, Ramírez-Wong B, Robles-Sánchez RM, Robles-Zepeda RE, González-Aguilar GA, Gutiérrez-Dorado R. Effect of extrusion conditions and the optimization of phenolic compound content and antioxidant activity of wheat bran using response surface methodology. *Plant Foods Hum Nutr.* (2018) 73:228-34. doi: 10.1007/s11130-018-0679-9
35. Sharma P, Gujral HS. Effect of sand roasting and microwave cooking on antioxidant activity of barley. *Food Res Int.* 44:235–40. doi: 10.1016/j.foodres.2010.10.030
36. Xu B, Chang SKC. Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *J Agric Food Chem.* (2008) 56:7165–75. doi: 10.1021/jf8012234
37. Arts MJTJ, Haenen GRMM, Wilms LC. Interactions between flavanoids and proteins: effect on the total antioxidant capacity. *J Agricult Food Chem.* (2002) 50:1184–7. doi: 10.1021/jf010855a
38. Zhu F, Cai YZ, Bao J, Corke H. Effect of γ-irradiation on phenolic compounds in rice grain. *Food Chem.* (2010) 120:74–77. doi: 10.1016/j.foodchem.2009.09.072
39. Stojceska V, Ainsworth P. The effect of different enzymes on the quality of high-fibre enriched brewer's spent grain breads. *Food Chem.* (2008) 110:865–72. doi: 10.1016/j.foodchem.2008.02.074
40. Stojceska V, Ainsworth P, Plunkett A, Ibanoglu S. The recycling of brewer's processing by-product into ready-to-eat snacks using extrusion technology. *J Cereal Sci.* (2008) 47:469–79. doi: 10.1016/j.jcs.2007.05.016
41. Altan A, Mccarthy KL, Maskan M. Evaluation of snack foods from barley-tomato pomace blends by extrusion processing. *J Food Eng.* (2008) 84:231–42. doi: 10.1016/j.jfoodeng.2007.05.014

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The Association Between Fasting Blood Sugar and Index of Nutritional Quality in Adult Women

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Aim: It's unclear whether diet quality affects glycemic management. The index of nutritional quality (INQ) can examine diets both quantitatively and qualitatively (INQ). Hence, this study aimed to determine whether INQ and fasting blood sugar (FBS) are related among Iranian women.

Methods: This cross-sectional study was conducted on 360 adult Iranian women. Data were collected on the participants' general characteristics, medical history, anthropometric indices, physical activity, and dietary intake. For nutrient intake assessment, a valid food frequency questionnaire (FFQ) was used, and INQ was then calculated using the daily nutrient intake.

Results: After adjusting for age, FBS was significantly inverse associated with INQ for vitamins A (B = −0.193, $p < 0.01$), magnesium (B = −0.137, $p < 0.01$), phosphor (B = −0.175, $p < 0.01$), zinc (B = −0.113, $p < 0.01$), vitamin K (B = −0.197, $p < 0.01$), manganese (B = −0.111, $p < 0.01$) and selenium (B = −0.123, $p < 0.01$). The association between FBS and INQ for Se and Mn was disappeared after further adjustment for gender, body mass index (BMI), menopausal status, and total energy intake.

Conclusion: There was a significant inverse relationship between FBS and the INQ of vitamin A, manganese, phosphor, zinc, vitamin K, magnesium, and selenium. Prospective cohort studies should be conducted to establish a causal relationship between FBS and INQ.

Keywords: index of nutritional quality, fasting blood sugar, dietary intake, glycemic control, medical history

INTRODUCTION

Hyperglycemia is observed in about one-fifth to one-fourth of the adult population in developing countries (1). Hyperglycemia is defined as a fasting blood glucose level of more than 126 mg/dl or a random blood glucose level of more than 200 mg/dl, both of which are common among hospitalized patients (2, 3). The development of hyperglycemia is linked to an increased risk of mortality (4) and infections in hospitalized patients, according to extensive data from observational studies (5). Some studies reported that correction of hyperglycemia with insulin administration reduces hospital complications and decreases mortality in cardiac diseases (6).

Dietary components may have significant effects on the management of hyperglycemia. Recently, several indicators were introduced to evaluate the quality of the diet. For example, glycemic index (GI) is a value used to measure how much specific foods increase blood sugar levels, and foods with a low GI < 50 cause a slower rise in blood glucose concentration compared to an equal carbohydrate amount from high GI foods (7). Evidence suggests that dietary management with a low GI diet improves glycemic control in diabetic patients (7, 8). Although carbohydrate is likely the most significant component of food to affect postprandial glycemia, previous research found that dietary fat and protein have a key role in the glycemic response following the ingestion of carbohydrate (9). Dietary fat can delay hyperglycemic peak responses by slowing glucose absorption (10–12) and dietary protein facilitates glucose clearance by stimulating insulin release (13–16). A decreased incidence of hyperglycemia was linked to a 'healthy traditional' dietary pattern rich in vegetables, grains, and products, fish, and shrimp. Greater consumption of fruits, juice, and alcohol, on the other hand, was linked to a higher risk of hyperglycemia (17–19). Controversial results in relation to hyperglycemia and dietary components were previously observed. For example, some studies reported that fat intake is positively associated with the prevalence of impaired fasting glucose (20, 21). While other studies did not highlight an association between total fat intake and the risk of type-2 diabetes mellitus (T2DM) (22–25).

The index of nutritional quality (INQ) score was created to assess the diet quality and comprises four components: variety, adequacy, moderation, and overall balance (26) and was used in a few studies (27–29). Previous studies in European adults have reported an inverse association between INQ and cardiovascular risk factors, including lipid biomarkers and obesity (30). The incidence of fasting blood sugar disorder was reported to decrease by 75% in men with higher scores of INQ (31). In another study, the patients in the highest tertile of INQ had fewer fasting blood sugar amounts. However, no significant correlations were observed in some studies between dietary quality indices and fasting blood sugar (29). For example, in a cross-sectional study on the correlation between diet quality and glycemic status in patients with type 2 diabetes, no significant correlations were observed between INQ and fasting blood sugar, glycosylated hemoglobin (HbA1c), insulin, and insulin resistance [28]. To the best of our knowledge, no firm association between INQ and FBS has been yet established. So, this study aimed to investigate the association between INQ and FBS in Iranian adults.

METHODS

This cross-sectional study was performed on 360 Iranian adult women in Tehran, Iran. The participants were selected from healthy women referring to the nutrition clinic of Shohadaye Tajrish Hospital, Tehran, Iran. The sample size was calculated using the OPENEPI software and the quantity of odds ratio (OR) acquired in a prior research [19]. The inclusion criteria were willingness to participate in the study, age between 35 and 75 years old, having no history of metabolic syndrome, not suffering from diseases affecting blood sugar, and did not use antihyperglycemic drugs. Participants with alcohol or drug addiction, have weight-related illnesses including specific psychological or neurological disorders, insulin resistance, thyroid disease, liver disease, kidney failure, infectious diseases, history of multiple sclerosis, hypertension, dialysis, and pregnant or lactating women were excluded from the study ($n = 7$). The objectives of the study were explained to the participants, and a written consent form was collected. Data on age, height, weight, and BMI were collected through face-to-face interviews and the amount of physical activity was estimated using a validated International Physical Activity Questionnaire (IPAQ).

FBS Measurement

Five ml of blood samples were collected from all participants after 10–12 hr of overnight fasting. In order to prevent glycolysis, plasma was isolated up to 1 h after sampling, and blood glucose levels were measured using glucose oxidase and photometry using the colorimetric method GOD-PAP solution (Pars Azmoun, Iran) and RA-1000 auto analyzer.

Dietary Assessment

A semi-quantitative food frequency questionnaire (FFQ) that has previously been validated in Iran was used to collect the necessary nutritional data [6]. The FFQ consisted of 147 food items with standard serving sizes commonly used by Iranians. Participants were asked to report the frequency of consumption of each food item according to its standard portion size during the last year. Final portion sizes were changed into g/day using household measures based on USDA database with minor modification for the special national foods like breads and the average. Then, the data obtained from these questionnaires were analyzed using nutritionist-IV software (version 4.1; First Databank Division; Hearst) and daily intake of energy and nutrients was calculated.

The INQ score assess variety, adequacy, moderation, and overall balance of the diet, thus it may capture different aspects of diet quality related to under- and over-nutrition (26).

The INQ analyzes foods, meals, and diets quantitatively and qualitatively and compares people's dietary intakes extracted from the FFQ with the recommended standards. It modifies the effect of total energy intake and provides accurate estimations of individual intake (32). The following nutrients were used in computing INQ: vitamin A, riboflavin, vitamin C, vitamin D, vitamin K, vitamin B6, thiamin, niacin, biotin, folate, vitamin

TABLE 1 | Characteristics of the participants with high and normal levels of fasting blood sugar.

Parameters	Normal FBS (n = 219)	High FBS (n = 134)	P
Age (y)	47.40 (± 7.4)	52.9 (± 8.7)	0.001
Height (cm)	156.292(± 5.6)	155.970(± 5.3)	0.590
Weight (kg)	69.187(± 10.8)	74.746(± 11.8)	0.000
Body mass index (BMI) (kg/m^2)	28.3012(± 4.08)	30.6590(± 4.17)	0.000
Physical activity (kcal/kg/h)	1.5646(± 1.5)	1.3769(± 1.4)	0.257
Smoking n (%)	1(0.5%)	2(1.5%)	0.56
Using alcohol n (%)	2 (0.9%)	1(0.7%)	0.68

B12, vitamin E, zinc (Zn), iron, copper, manganese (Mn), phosphor (P), manganese (Mg), and selenium (Se).

The INQ for each nutrient was then calculated as the ratio of the amount of nutrient consumed per 1,000 kcal per day to the recommended dietary allowance of the RDA for nutrients per 1,000 kcal (33). In cases where the RDA for specific nutrients was not defined, adequate intake (AI) values were used. Individuals whose average daily energy intake was reported to be <800 or >4,200 kcal or who did not consume more than 70 food items (>40% of items in the food frequency questionnaire) were excluded from the study.

Statistical Analysis

To compare different variables in individuals with normal and high FBS profiles, independent t-test and chi-square methods for quantitative and qualitative variables were used, respectively. A multiple linear regression method was used to investigate the association between fasting blood sugar and the INQ after adjusting for age (model 1), age, gender, BMI, menopausal status, and total energy intake (model 2). The SPSS software version 23 was used for statistical analysis and the probability level of $P < 0.05$ was considered statistically significant.

RESULTS

The general characteristics of the participants are presented in **Table 1**. People with higher FBS (n = 134) had significantly higher age (52.9 ± 8.7 vs. 47.40 ± 7.4, P = 0.001), weight (74.74 ± 11.8 vs. 69.18 ± 10.8, P = 0.001) and BMI (30.65 ± 4.17 vs. 28.30 ± 4.08, P = 0.001) compared with the participants with normal FBS (n = 219). No significant difference was observed between the two groups regarding height, physical activity, smoking, and using alcohol.

Table 2 shows the association between dietary intake among people with normal and high levels of FBS. People with higher FBS (n = 134) had significantly higher intake of thiamin (2.51 ± 1.06 vs. 2.05 ± 0.63, P=0.015), riboflavin (2.48 ± 1.55 vs. 1.95 ± 0.82, P = 0.046), niacin (25.37 ± 8.60 vs. 22.18 ± 5.95, P = 0.051), vitamin B6 (1.93 ± 0.87 vs. 1.60 ± 0.51, P = 0.032), folate (704.06 ± 205.20 vs. 612.48 ± 194.57, P= 0.051), vitamin B12 (4.41 ± 4.43 vs. 2.96 ± 1.41, P = 0.028), biotin (33.45 ± 16.15 vs. 26.67 ± 10.55, P = 0.024), P (1,567.48 ± 1,094.6 vs. 1,217.70 ± 492.78, P = 0.047), selenium (105.31 ± 45.22 vs. 85.69 ± 26.28, P = 0.014) compared with the participants with normal FBS.

Table 3 presents the association between FBS and the INQ of nutrients. There was a significant inverse association between FBS and the INQ of vitamin A (B = −0.193, P < 0.01), Magnesium (B = −0.137, P < 0.01), P (B = −0.175, P < 0.01), zinc (B = −0.113, P < 0.01), vitamin K (B = −0.197, P < 0.01), Mn (B = −0.111, P < 0.01) and Se (B = −0.123, P < 0.01) after adjustment for age (model 1). The association between the FBS and INQ of Se and Mn were disappeared after further adjustment for gender, BMI, menopausal status, and total energy intake (Model 2).

DISCUSSION

The present study investigated the association between FBS and INQ in adult women. There was a significant difference between the INQ of vitamin A, Mg, zinc, vitamin K, Mn, and selenium with FBS after adjustments for age, gender, BMI, menopausal status, and total energy intake.

Similar to this study, some studies found that vitamin A has both antioxidant and antihyperglycemic potential and, therefore, can be considered a hypoglycemic factor (34, 35). Vitamin A can impact T2DM pathogenesis through several potential molecular mechanisms, including chelation of oxide radicals, improves insulin sensitivity, and beta-cell regeneration (36). Shidfar et al., on the other hand, discovered no correlation between FBS and vitamin A in 48 diabetes mellitus type 1 (DMTI) patients (37). Jafarirad et al. showed in another trial that vitamin A had no significant influence on lipid profiles, FBS, or liver enzymes (38). The most likely explanation for these discrepancies is that the current research employed an assessment of nutritional quality rather than the quantity of nutrients consumed (34).

In line with this study, some studies reported the beneficial effects of Mg on glycemic control in individuals with T2DM (39–42). While, other studies showed no significant effects of Mg on T2DM (39, 40). The improvement in the glycemic control indicators after Mg therapy could be explained by different mechanisms, including the influence of Mg on insulin receptor activity through enhanced tyrosine kinase phosphorylation (43, 44). There was the possibility that Mg could help facilitate the translocation of glucose transporter number 4 (GLUT 4) to the cell membrane caused by the activation of tyrosine–kinase in the presence of Mg (45).

Regarding the association of FBS and dietary phosphorus, Fang et al. reported that the serum level of phosphate in the type 2 diabetic group was significantly lower than that in the control group (46). Besides, Duan et al. demonstrated that higher urinary phosphorus excretion was associated with decreased risk of T2DM (47). However, other research found that phosphorus had no significant influence on T2DM (48). For instance, one research discovered that diabetic rats also had increased plasma phosphorus amounts (49). The reason for the difference in results may be related to the study population and some of which were performed on animals (49). Phosphorus concentration

TABLE 2 | Dietary intakes among people with normal and high levels of fasting blood sugar.

Parameters	Normal FBS	High FBS	P
Total energy intake (Kcal/d)	2,473.36 (± 703.65)	2,869.39 (± 997.91)	0.039
Protein (gr/d)	75.67 (± 27.52)	92.10 (± 46.31)	0.45
Carbohydrate (gr/d)	365.51 (± 96.05)	420.47 (± 133.72)	0.34
Fat (gr/d)	84.86 (± 36.46)	96.91 (± 44.66)	0.191
Cholesterol (mg/dl)	216.19 (± 110.84)	254.93 (± 137.90)	0.170
Saturated fatty acids (mg/dl)	25.64 (± 11.97)	31.124 (± 21.40)	0.137
Mono unsaturated fatty acids (mg/dl)	29.292413 (± 14.26)	31.097204 (± 12.94)	0.576
Poly unsaturated fatty acids (mg/dl)	19.653896 (± 10.29)	19.293867 (± 7.65)	0.871
PFA3 (mg/dl)	1.241493 (± 0.64)	1.259295 (± 0.514)	0.899
PFA6 (mg/dl)	6.074097 (± 8.48)	5.141801 (± 6.17)	0.608
Sodium (IU/L)	5,115.400291 (± 2302.7)	5,934.210146 (± 2656.7)	0.150
Potassium (IU/L)	3,652.394859 (± 1477.40)	4,298.316661 (± 1958.69)	0.096
Vitamin A (IU/L)	505.368241 (± 288.28)	444.731172 (± 209.94)	0.277
Beta-carotene (IU/L)	2,924.71 (± 1354.95)	3,212.54 (± 2216.34)	0.464
Alpha-carot (IU/L)	438.97 (± 376.75)	595.76 (± 691.5)	0.183
Lutein (IU/L)	1,573.75 (± 904.35)	1,541.09 (± 742.75)	0.869
Beta-cryptoxanthin (IU/L)	322.25 (± 201.73)	284.90 (± 163.59)	0.400
Lycopene (IU/L)	7,173.65 (± 4313.99)	7,843.467731(± 4,733.68)	0.519
Vitamin C (IU/L)	138.61 (± 138.61)	156.78 (± 156.78)	0.401
Vitamin E (IU/L)	17.96 (± 14.43)	17.546 (± 9.76)	0.892
Alpha-tocopherol (IU/L)	11.841 (± 9.40)	11.48 (± 6.70)	0.858
Thiamin (IU/L)	2.05 (± 0.637)	2.51(± 1.06)	0.015
Riboflavin (IU/L)	1.957 (± 0.829)	2.48 (± 1.55)	0.046
Niacin (IU/L)	22.188 (± 5.95)	25.37 (± 8.60)	0.051
Vitamin B6 (IU/L)	1.60 (± 0.51)	1.93 (± 0.87)	0.032
Folate (IU/L)	612.48 (± 194.57)	704.06 (± 205.20)	0.051
Folate (IU/L)	755.47 (± 237.27)	856.65 (± 301.00)	0.098
Vitamin B12 (IU/L)	2.96 (± 1.41)	4.41 (± 4.43)	0.028
Biotin (IU/L)	26.67 (± 10.55)	33.45(± 16.15)	0.024
Pantothenic (IU/L)	4.71 (± 1.81)	5.61 (± 3.00)	0.092
Vitamin K (IU/L)	127.47 (± 56.87)	118.18 (± 51.52)	0.455
Phosphor (IU/L)	1,567.48 (± 1,094.6)	1,217.70 (± 492.78)	0.047
Magnesium (IU/L)	401.01 (± 169.82)	344.61 (± 131.99)	0.099
Zinc (IU/L)	11.77 (± 6.19)	10.54 (± 5.73)	0.372
Copper (IU/L)	1.79 (± 0.51)	1.97 (± 0.64)	0.167
Manganese (IU/L)	6.06 (± 2.93)	5.18 (± 1.80)	0.091
Selenium (IU/L)	105.31 (± 45.22)	85.69 (± 26.28)	0.014
Iron (mg/dl)	2,956.16 (± 1,620.08)	3,584.31 (± 1,980.78)	0.126
Chromium (IU/L)	014 (± 0.047)	026 (± 0.104)	0.442
Fiber-t (mg/dl)	27.41 (± 10.74)	30.073 (± 11.58)	0.302
Fiber-s (mg/dl)	1.02 (± 4.29)	1.07 (± 0.92)	0.768
Fiber-is (mg/dl)	11.53 (± 6.62)	5.59 (± 4.29)	0.484
Crude-fiber (mg/dl)	11.128 (± 6.82)	11.53 (± 6.62)	0.797
Sugar-t (mg/dl)	128.49 (± 46.73)	150.25 (± 70.52)	0.096
Glucose (mg/dl)	20.30 (± 8.10)	22.096 (± 9.33)	0.369
Gal (mg/dl)	2.69 (± 2.42)	5.09 (± 10.66)	0.114
Fructose (mg/dl)	25.98 (± 10.26)	27.67 (± 11.62)	0.499
Sucrose (mg/dl)	46.40 (± 21.12)	50.49 (± 29.76)	0.471
Lactose (mg/dl)	10.13 (± 7.12)	17.21 (± 28.84)	0.088
Maltose (mg/dl)	2.73 (± 1.23)	3.11 (± 1.68)	0.235
Caffeine (mg/dl)	161.00 (± 94.46)	200.91 (± 117.54)	0.095

(Continued)

TABLE 2 | Continued

TABLE 3 | The association between the index of nutritional quality (INQ) of the nutrients and FBS.

INQ	Model 1[a]		Model 2[a]	
	B	*P*-value	B	*P*-value
Vitamin A (IU/L)	−0.193	<0.01	−0.227	<0.01
Mg (IU/L)	−0.137	<0.01	−0.153	<0.01
P (IU/L)	−0.175	<0.01	−0.236	<0.01
Zn (IU/L)	−0.113	0.02	−0.192	<0.01
Copper (IU/L)	−0.018	0.72	0.088	0.16
Mn (IU/L)	−0.111	0.02	0.057	0.37
Se (IU/L)	−0.123	<0.01	−0.097	0.12
E (IU/L)	−0.017	0.71	0.020	0.74
B1 (IU/L)	−0.032	0.50	−0.057	0.36
B2 (IU/L)	0.072	0.13	0.064	0.31
B3 (IU/L)	0.003	0.94	0.049	0.43
B6 (IU/L)	0.051	0.28	0.033	0.60
B9 (IU/L)	0.027	0.57	0.047	0.45
B12 (IU/L)	0.020	0.67	0.038	0.54
B5 (IU/L)	0.058	0.22	0.132	0.05
Biotin (IU/L)	−0.016	0.73	−0.010	0.87
Vitamin C (IU/L)	0.051	0.28	0.055	0.38
Vitamin D (IU/L)	0.037	0.43	−0.005	0.93
Vitamin K (IU/L)	−0.197	<0.01	−0.165	<0.01

[a]*First model adjusted for age and the second model adjusted for age, gender, BMI, menopausal status, and total energy intake.*

is a determining factor in regulating the metabolism and rate of oxygen consumption. In diabetes, the highest oxygen consumption is associated with the lowest concentration of phosphorus (50).

In terms of the association between of FBS and Zn, a negative association was found between serum Zn with FBS. Low Zn levels were reported to be associated with poor glycemic control and poor glycemic control is a strong predictor of Zn deficiency (51). Another study reported that there is no definite cause-and-effectrelationship between Zn and the level of FBS (52). The reduced concentration of Zn in T2DM was indicated by Saharia and Goswami (53), which was in line with the present study. Al-Maroof and Al-Sharbatti (54) also reported that Zn levels were lower in diabetic patients compared to the controls, and a strong negative relationship was found between glycosylated hemoglobin levels of diabetic patients with their serum Zn levels. Zn has an important role in the utilization of glucose by muscle and fat cells (55). Zn acts as a cofactor for intracellular enzymes involved in protein, lipid, and glucose metabolism (55). Zinc is required for the stability of insulin hexamers and the hormone's pancreatic storage (56).

Similar with the results of the present study, high circulating levels of vitamin K were reported to be related to a lower risk of the high amount of FBS (57). Some studies found a lower risk for diabetes mellitus in people with higher vitamin K intakes (58, 59). Rees et al. (60) in a systematic review of studies that evaluated the association of vitamin K deficiency with T2DM concluded that there is no evidence of an effect of vitamin K and a higher level of FBS. The differences in the obtained results can be related to the study population since some studies have been done on diabetics and some others on healthy people. Vitamin K has been shown to reduce insulin resistance by inhibiting inflammation. Vitamin K may inhibit the generation of IL-6 (Interleukin 6) in lipopolysaccharide-induced inflammatory models (61, 62). Moreover, high plasma Vitamin K concentrations were associated with decreased concentrations of inflammatory markers TNF-α (Tumor necrosis factor alpha) and IL-6 (63). In a study that assessed the status of fat-soluble vitamins in patients with chronic pancreatitis, the results indicated that the serum concentrations of fat-soluble vitamins were decreased in these patients (64).

Tan et al. revealed that pregnant women with impaired glucose tolerance (IGT) or gestational diabetes mellitus (GDM) had lower blood selenium levels and discovered an inverse association between FBS and serum selenium levels (65), which was in line with the present study. However, the evidence for a link between selenium and GDM is inconsistent, with other research finding no correlation between selenium concentration and FBS level (66, 67).

Moreover, a higher intake of manganese was frequently reported to be associated with a lower level of FBS (68–70). However, another study found that both low and high levels of plasma manganese were associated with higher levels of FBS (71). Manganese plays significant roles in multiple physiological functions, including glucose and lipid metabolism, insulin production, and insulin secretion. Manganese deficiency leads to impaired glucose tolerance and increased risk of metabolic syndrome through impaired glucose and lipid metabolism (72, 73).

To the best of our knowledge, few studies investigated the association between FBS and the quality of diet. The strengths of the present study were its acceptable sample size and using the index of nutritional quality. However, the results may be affected by some limitations. This study was a cross-sectional study, which could not explain the causal relationship. Future prospective studies should be performed to establish a causal relationship between FBS and the INQ in adult women. Moreover, this study was performed on young women and cannot be generalized to the public. In addition, the semi-quantitative FFQ is not the best indicator to know the amount of micronutrients that are being ingested and, probably, it would be more indicated to carry out an intake analysis with double weighing in future studies.

The present study provides the first evidence for an association between FBS and the INQ in adult women. According to the findings of the study, there was a significant inverse association between FBS and the INQ of vitamin A, Mg, P, zinc, vitamin K, Mn, and Se. Prospective cohort studies should be performed to establish a causal relationship between FBS and INQ in adult women.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by IR.SBMU.nnftri.Rec.1400.049. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FA, MG, SD, GM, MA, HS, AA, MM, and SD designed the study, involved in the data collection, analysis, and drafting of the manuscript. SD and FV were involved in the design of the study, analysis of the data, and critically reviewed the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Gosmanov AR, Umpierrez GE. Management of hyperglycemia during enteral and parenteral nutrition therapy. *Curr Diab Rep.* (2013) 13:155–62. doi: 10.1007/s11892-012-0335-y
2. Umpierrez GE, Isaacs SD, Bazargan N, You X, Thaler LM, Kitabchi AE. Hyperglycemia: an independent marker of in-hospital mortality in patients with undiagnosed diabetes. *J Clin Endocrinol Metab.* (2002) 87:978–82. doi: 10.1210/jcem.87.3.8341
3. Cook CB, Kongable GL, Potter DJ, Abad VJ, Leija DE, Anderson M. Inpatient glucose control: a glycemic survey of 126 US hospitals. *J Hosp Med.* (2009) 4:E7–14. doi: 10.1002/jhm.533
4. Cook A, Burkitt D, Mcdonald L, Sublett L. Evaluation of glycemic control using NPH insulin sliding scale versus insulin aspart sliding scale in continuously tube-fed patients. *Nutr Clin Pract.* (2009) 24:718–22. doi: 10.1177/0884533609351531
5. Pleva M, Mirtallo JM, Steinberg SM. Hyperglycemic events in non–intensive care unit patients receiving parenteral nutrition.

Nutr Clin Pract. (2009) 24:626–34. doi: 10.1177/0884533609339069

6. Furnary AP, Gao G, Grunkemeier GL, Wu Y, Zerr KJ, Bookin SO, et al. Continuous insulin infusion reduces mortality in patients with diabetes undergoing coronary artery bypass grafting. *J Thorac Cardiovasc Surg.* (2003) 125:1007–21. doi: 10.1067/mtc.2003.181
7. Jenkins DJ, Wolever T, Taylor RH, Barker H, Fielden H, Baldwin JM, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr.* (1981) 34:362–6. doi: 10.1093/ajcn/34.3.362
8. Schwingshackl L, Chaimani A, Hoffmann G, Schwedhelm C, Boeing H. Impact of different dietary approaches on glycemic control and cardiovascular risk factors in patients with type 2 diabetes: a protocol for a systematic review and network meta-analysis. *Syst Rev.* (2017) 6:1–7. doi: 10.1186/s13643-017-0455-1
9. Sheard NF, Clark NG, Brand-Miller JC, Franz MJ, Pi-Sunyer FX, Mayer-Davis E, et al. Dietary carbohydrate (amount and type) in the prevention and management of diabetes: a statement by the American Diabetes Association. *Diabetes Care.* (2004) 27:2266–71. doi: 10.2337/diacare.27.9.2266
10. Collier G, O'dea K. The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein. *Am J Clin Nutr*. (1983) 37:941–44. doi: 10.1093/ajcn/37.6.941
11. Collier G, Mclean A, O'dea, K. Effect of co-ingestion of fat on the metabolic responses to slowly and rapidly absorbed carbohydrates. *Diabetologia*. (1984) 26:50–4. doi: 10.1007/BF00252263
12. Nuttall FQ, Gannon MC. Plasma glucose and insulin response to macronutrients in nondiabetic and NIDDM subjects. *Diabetes Care.* (1991) 14:824–38. doi: 10.2337/diacare.14.9.824
13. Floyd JC, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW. Synergistic effect of essential amino acids and glucose upon insulin secretion in man. *Diabetes.* (1970) 19:109–15. doi: 10.2337/diab.19.2.109
14. Nuttall FQ, Mooradian AD, Gannon MC, Billington C, Krezowski P. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. *Diabetes Care.* (1984) 7:465–70. doi: 10.2337/diacare.7.5.465
15. Van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am J Clin Nutr.* (2000) 72:96–105. doi: 10.1093/ajcn/72.1.96
16. Doaei S, Kalantari N, Izadi P, Salonurmi T, Mosavi Jarrahi A, Rafieifar S, et al. Changes in FTO and IRX3 gene expression in obese and overweight male adolescents undergoing an intensive lifestyle intervention and the role of FTO genotype in this interaction. *J Transl Med.* (2019) 17:176. doi: 10.1186/s12967-019-1921-4
17. Hong X, Xu F, Wang Z, Liang Y, Li J. Dietary patterns and the incidence of hyperglyacemia in China. *Public Health Nutr.* (2016) 19:131–41. doi: 10.1017/S1368980015000774
18. Ashkar F, Rezaei S, Salahshoornezhad S, Vahid F, Gholamalizadeh M, Dahka SM, et al. The Role of medicinal herbs in treatment of insulin resistance in patients with polycystic ovary syndrome: a literature review. *Biomol Concepts.* (2020) 11:57–75. doi: 10.1515/bmc-2020-0005
19. Vahid F, Hekmatdoost A, Mirmajidi S, Doaei S, Rahmani D, Faghfoori Z. Association between index of nutritional quality and nonalcoholic fatty liver disease: the role of vitamin D and B group. *Am J Med Sci*. (2019) 358:212–8. doi: 10.1016/j.amjms.2019.06.008
20. Feskens EJ, Virtanen SM, Räsänen L, Tuomilehto J, Stengård J, Pekkanen J, et al. Dietary factors determining diabetes and impaired glucose tolerance: a 20-year follow-up of the Finnish and Dutch cohorts of the seven countries study. *Diabetes Care.* (1995) 18:1104–12. doi: 10.2337/diacare.18.8.1104
21. Narasimhan S, Nagarajan L, Vaidya R, Gunasekaran G, Rajagopal G, Parthasarathy V, et al. Dietary fat intake and its association with risk of selected components of the metabolic syndrome among rural South Indians. *Indian J Endocrinol Metab.* (2016) 20:47. doi: 10.4103/2230-8210.172248
22. Hu FB, Van Dam R, Liu S. Diet and risk of type II diabetes: the role of types of fat and carbohydrate. *Diabetologia.* (2001) 44:805–17. doi: 10.1007/s001250100547
23. Meyer KA, Kushi LH, Jacobs DR, Folsom AR. Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care.* (2001) 24:1528–35. doi: 10.2337/diacare.24.9.1528
24. Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, et al. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr.* (2001) 73:1019–26. doi: 10.1093/ajcn/73.6.1019
25. Guasch-Ferré M, Becerra-Tomas N, Ruiz-Canela M, Corella D, Schröder H, Estruch R, et al. Total and subtypes of dietary fat intake and risk of type 2 diabetes mellitus in the Prevención con Dieta Mediterránea (PREDIMED) study. *Am J Clin Nutr*. (2017) 105, 723–735. doi: 10.3945/ajcn.116.142034
26. Behrad Nasab M, Afsharfar M, Ahmadzadeh M, Vahid F, Gholamalizadeh M, Abbastorki S, et al. Comparison of the index of nutritional quality in breast cancer patients with healthy women. *Front Nutr*. (2022) 9:811827. doi: 10.3389/fnut.2022.811827
27. Asghari G, Mirmiran P, Rashidkhani B, Asghari-Jafarabadi M, Mehran M, Azizi F. The association between diet quality indices and obesity: tehran lipid and glucose study. *Arch Iran Med.* (2012) 15:599–605.
28. Asghari G, Mirmiran P, Hosseni-Esfahani F, Nazeri P, Mehran M, Azizi F. Dietary quality among Tehranian adults in relation to lipid profile: findings from the Tehran lipid and glucose study. *J Health Popul Nutr.* (2013) 31:37–48. doi: 10.3329/jhpn.v31i1.14747
29. Daneshzad E, Larijani B, Azadbakht L. Diet quality indices and cardiovascular diseases risk factors among diabetic women. *J Sci Food Agric.* (2019) 99:5926–33. doi: 10.1002/jsfa.9867
30. Lassale C, Gunter MJ, Romaguera D, Peelen LM, Van Der Schouw YT, Beulens JW, et al. Diet quality scores and prediction of all-cause, cardiovascular and cancer mortality in a pan-European cohort study. *PLoS ONE.* (2016) 11:e0159025. doi: 10.1371/journal.pone.0159025
31. Gopinath B, Rochtchina E, Flood V, Mitchell P. Diet quality is prospectively associated with incident impaired fasting glucose in older adults. *Diabetic medicine.* (2013) 30:557–62. doi: 10.1111/dme.12109
32. Vahid F, Rahmani D, Davoodi SH, Hekmatdoost A. The association among maternal index of nutritional quality, dietary antioxidant index, and odds of miscarriage incidence: case-control study. *J Am Coll Nutr.* (2022) 41:310–7. doi: 10.1080/07315724.2021.1880987
33. Gholamalizadeh M, Rastgoo S, Doaei S, Vahid F, Malmir H, Ashoori N, et al. Index of nutritional quality (INQ) and the risk of obesity in male adolescents: a case-control study. *Biol Trace Elem Res.* (2021) 199:1701–6. doi: 10.1007/s12011-020-02297-3
34. Horváth M, Babinszky L. Impact of selected antioxidant vitamins (Vitamin A, E and C) and micro minerals (Zn, Se) on the antioxidant status and performance under high environmental temperature in poultry: A review. *Acta Agriculturae Scandinavica.* (2018) 68:152–60. doi: 10.1080/09064702.2019.1611913
35. Meerza D, Iqbal S, Zaheer S, Naseem I. Retinoids have therapeutic action in type 2 diabetes. *Nutrition.* (2016) 32:898–903. doi: 10.1016/j.nut.2016.02.003
36. Iqbal S, Naseem I. Role of vitamin A in type 2 diabetes mellitus biology: effects of intervention therapy in a deficient state. *Nutrition.* (2015) 31:901–7. doi: 10.1016/j.nut.2014.12.014
37. Shidfar F, Aghasi M, Vafa M, Heydari I, Hosseini S, Shidfar S. Effects of combination of zinc and vitamin A supplementation on serum fasting blood sugar, insulin, apoprotein B and apoprotein AI in patients with type I diabetes. *Int J Food Sci Nutr.* (2010) 61:182–91. doi: 10.3109/09637480903334171
38. Jafarirad S, Siassi F, Harirchian M-H, Amani R, Bitarafan S, Saboor-Yaraghi A. The effect of vitamin a supplementation on biochemical parameters in multiple sclerosis patients. *Iran Red Crescent Med J.* (2013) 15:194. doi: 10.5812/ircmj.3480
39. De Lourdes Lima M, Cruz T, Pousada JC, Rodrigues LE, Barbosa K, Canguçu V. The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. *Diabetes Care.* (1998) 21:682–6. doi: 10.2337/diacare.21.5.682
40. De Valk, H., Verkaaik, R., Van Rijn, H., Geerdink, R., and Struyvenberg, A. (1998). Oral magnesium supplementation in insulin-requiring Type 2 diabetic patients. *Diabetic Med.* 15, 503–7. doi: 10.1002/(SICI)1096-9136(199806)15:6<503::AID-DIA596>3.0.CO;2-M
41. Rodríguez-Morán M, Guerrero-Romero F. Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects: a randomized double-blind controlled trial. *Diabetes Care.* (2003) 26:1147–52. doi: 10.2337/diacare.26.4.1147

42. Solati M, Ouspid E, Hosseini S, Soltani N, Keshavarz M, Dehghani M. Oral magnesium supplementation in type II diabetic patients. *Med J Islam Repub Iran.* (2014) 28:67.
43. Paolisso G, Barbagallo M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. *Am J Hypertens.* (1997) 10:346–55. doi: 10.1016/S0895-7061(96)00342-1
44. Takaya J, Higashino H, Kobayashi Y. Intracellular magnesium and insulin resistance. *Magnesium Res.* (2004) 17:126–36.
45. Kolterman O, Gray R, Griffin J, Burstein P, Insel J, Scarlett J, et al. Receptor and postreceptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. *J Clin Invest.* (1981) 68:957–69. doi: 10.1172/JCI110350
46. Fang L, Li X. Level of serum phosphorus and adult type 2 diabetes mellitus. *J Cent South Univ Med Sci.* (2016) 41:502–6. doi: 10.11817/j.issn.1672-7347.2016.05.009
47. Duan S, Sun L, Zhu H, Nie G, Zhang C, Huang Z, et al. Association of urinary calcium and phosphorus excretion with renal disease progression in type 2 diabetes. *Diabetes Res Clin Pract.* (2021) 178:108981. doi: 10.1016/j.diabres.2021.108981
48. Vorum H, Ditzel J. Disturbance of inorganic phosphate metabolism in diabetes mellitus: its relevance to the pathogenesis of diabetic retinopathy. *J Ophthalmol.* (2014) 2014:135287. doi: 10.1155/2014/135287
49. Babu PS, Srinivasan K. Influence of dietary capsaicin and onion on the metabolic abnormalities associated with streptozotocin induced diabetes mellitus. *Mol Cell Biochem.* (1997) 175:49–57. doi: 10.1023/A:1006881027166
50. Ditzel J, Lervang H-H. Disturbance of inorganic phosphate metabolism in diabetes mellitus: its impact on the development of diabetic late complications. *Curr Diabetes Rev.* (2010) 6:323–33. doi: 10.2174/157339910793360833
51. Farooq DM, Alamri AF, Alwhahabi BK, Metwally AM, Kareem KA. The status of zinc in type 2 diabetic patients and its association with glycemic control. *J Family Community Med.* (2020) 27:29. doi: 10.29309/TPMJ/2020.27.10.4048
52. Chausmer AB. Zinc, insulin and diabetes. *J Am Coll Nutr.* (1998) 17:109–15. doi: 10.1080/07315724.1998.10718735
53. Saharia GK, Goswami RK. Evaluation of serum zinc status and glycated hemoglobin of type 2 diabetes mellitus patients in a tertiary care hospital of assam. *J Lab Physicians.* (2013) 5:30–3. doi: 10.4103/0974-2727.115923
54. Masood N, Baloch GH, Ghori RA, Memon IA, Memon MA, Memon MS. Serum zinc and magnesium in type-2 diabetic patients. *J Coll Physicians Surg Pak.* (2009) 19:483–6.
55. Salgueiro MJ, Krebs N, Zubillaga MB, Weill R, Postaire E, Lysionek AE, et al. Zinc and diabetes mellitus. *Biol Trace Elem Res.* (2001) 81:215–28. doi: 10.1385/BTER:81:3:215
56. Wijesekara N, Chimienti F, Wheeler M. Zinc, a regulator of islet function and glucose homeostasis. *Diabetes Obes Metab.* (2009) 11:202–14. doi: 10.1111/j.1463-1326.2009.01110.x
57. Zwakenberg SR, Remmelzwaal S, Beulens JW, Booth SL, Burgess S, Dashti HS, et al. Circulating phylloquinone concentrations and risk of type 2 diabetes: a mendelian randomization study. *Diabetes.* (2019) 68:220–5. doi: 10.2337/db18-0543
58. Beulens JW, Grobbee DE, Sluijs I, Spijkerman AM, Van Der Schouw YT. Dietary phylloquinone and menaquinones intakes and risk of type 2 diabetes. *Diabetes Care.* (2010) 33:1699–705. doi: 10.2337/dc09-2302
59. Ibarrola-Jurado N, Salas-Salvado J, Martinez-Gonzalez MA, Bullo M. Dietary phylloquinone intake and risk of type 2 diabetes in elderly subjects at high risk of cardiovascular disease. *Am J Clin Nutr.* (2012) 96:1113–8. doi: 10.3945/ajcn.111.033498
60. Comerford KB. Recent developments in multivitamin/mineral research. *Adv Nutr.* (2013) 4:644–56. doi: 10.3945/an.113.004523
61. Reddi K, Henderson B, Meghji S, Wilson M, Poole S, Hopper C, et al. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. *Cytokine.* (1995) 7:287–90. doi: 10.1006/cyto.1995.0034
62. Ohsaki Y, Shirakawa H, Hiwatashi K, Furukawa Y, Mizutani T, Komai M. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. *Biosci Biotechnol Biochem.* (2006) 70:926–32. doi: 10.1271/bbb.70.926
63. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB Sr, Dawson-Hughes B, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. *Am J Epidemiol.* (2008) 167:313–20. doi: 10.1093/aje/kwm306
64. Sikkens EC, Cahen DL, Koch AD, Braat H, Poley J-W, Kuipers EJ, et al. The prevalence of fat-soluble vitamin deficiencies and a decreased bone mass in patients with chronic pancreatitis. *Pancreatology.* (2013) 13:238–42. doi: 10.1016/j.pan.2013.02.008
65. Tan M, Sheng L, Qian Y, Ge Y, Wang Y, Zhang H, et al. Changes of serum selenium in pregnant women with gestational diabetes mellitus. *Biol Trace Elem Res.* (2001) 83:231–7. doi: 10.1385/BTER:83:3:231
66. Al-Saleh E, Nandakumaran M, Al-Shammari M, Al-Harouny A. Maternal–fetal status of copper, iron, molybdenum, selenium and zinc in patients with gestational diabetes. *J Matern Fetal Neonatal Med.* (2004) 16:15–21. doi: 10.1080/14767050412331283139
67. Bo S, Lezo A, Menato G, Gallo M-L, Bardelli C, Signorile A, et al. Gestational hyperglycemia, zinc, selenium, and antioxidant vitamins. *Nutrition.* (2005) 21:186–91. doi: 10.1016/j.nut.2004.05.022
68. Burlet E, Jain SK. Manganese supplementation reduces high glucose-induced monocyte adhesion to endothelial cells and endothelial dysfunction in Zucker diabetic fatty rats. *J Biol Chem.* (2013) 288:6414–09. doi: 10.1074/jbc.M112.447805
69. Juttukonda LJ, Berends ETM, Zackular JP, Moore JL, Stier MT, Zhang Y, et al. Dietary manganese promotes staphylococcal infection of the heart. *Cell Host Microbe.* (2017) 22: 531–42.e8. doi: 10.1016/j.chom.2017.08.009
70. Gong JH, Lo K, Liu Q, Li J, Lai S, Shadyab AH, et al. Dietary manganese, plasma markers of inflammation, and the development of type 2 diabetes in postmenopausal women: findings from the women's health initiative. *Diabetes Care.* (2020) 43:1344–51. doi: 10.2337/dc20-0243
71. Shan Z, Chen S, Sun T, Luo C, Guo Y, Yu X, et al. U-shaped association between plasma manganese levels and type 2 diabetes. *Environ Health Perspect.* (2016) 124:1876–81. doi: 10.1289/EHP176
72. Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. *Mol Aspects Med.* (2005) 26:353–62. doi: 10.1016/j.mam.2005.07.003
73. Li L, Yang X. The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. *Oxid Med Cell Longev.* (2018) 2018:7580707. doi: 10.1155/2018/7580707

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Organic Egg Consumption: A Systematic Review of Aspects Related to Human Health

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Consumption of organic foods has increased recently, but evidence about their potential health benefits is still limited. This systematic review aims to synthesize the available scientific evidence on the association between organic egg consumption and human health. We searched for peer-reviewed articles on this subject indexed in the MEDLINE, EMBASE, Web of Science and Cochrane Library databases from the inception date to April 13, 2022. This review was based on PRISMA guideline recommendations. Three studies on organic egg consumption in humans were included. After 8 weeks of consuming organic eggs, one randomized crossover trial found that participants had higher serum concentrations of the beta-carotene lutein compared to the period without consuming organic eggs. Moreover, in a cross-sectional study with nationally representative data from Americans over the age of 50, it was found that consumption of organic eggs was associated with lower levels of the inflammatory markers C-reactive protein and cystine C compared with conventional eggs. Finally, in a cohort of children aged 0 to 2 years, no significant association was observed between consuming organic eggs and the risk of eczema. In conclusion, the evidence about the potential benefits of organic egg consumption and human health is promising but still requires further research. A human research agenda is proposed based on laboratory studies pointing out that organic eggs have a more desirable nutritional profile than conventional eggs.

Keywords: chicken eggs, health benefits, systematic review, organic food attributes, dietary pattern

INTRODUCTION

There has been a recent increase in the consumption of organic food (1), particularly in developed countries, which may be due to the consumer's perception of the potential effect of this type of production related to sustainability, animal welfare and, especially, of their perception that organic products are healthier than those produced conventionally (1–5). The first two aspects are regulated by the competent authorities that define objective criteria to be applied with respect to the products (e.g., pesticides authorized at certain levels) and processes (e.g., conditions that ensure the natural behavior of the animals) used (6, 7). On the other hand, the concrete effects of organic foods on health depend on being verified in scientific studies, which are still scarce and provide contradictory results (7, 8). It should also be considered that the inconsistent findings on the health effect of the predominantly organic consumption pattern can be explained because the effects may vary according to each food consumed, although to a small extent (9–11).

Among the specific foods included in worldwide dietary patterns that are organically produced is the chicken egg. Eggs are a complete food, providing proteins of high biological value, unsaturated fatty acids, vitamins and minerals with antioxidant potential, and are also widely consumed worldwide due to their affordable market price (12, 13). The production of organic eggs is regulated in Europe and the United States (US) and requires that hens receive feed from organic vegetables that are not only cage-free but also have an outdoor area to move and behave as freely as they did originally (14–16). It is important to note that although there is no consensus yet, biochemical trials have assessed organic eggs as having lower concentrations of environmental contaminants and higher concentrations of micronutrients desirable for their positive health effects (17–20).

Considering that the emerging knowledge on specific organic foods is still in an initial phase, this systematic review was proposed with the aim of synthesizing the available evidence on the association between organic egg consumption and human health. In addition, comments on the research agenda about the proposed topic are presented.

METHODS

A systematic review of the literature was carried out based on the recommendations of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) (21) guidelines. The protocol of this review was registered in the PROSPERO database (registration number: CRD42022328052).

We searched the MEDLINE, EMBASE, Web of Science, and Cochrane Library databases for peer-reviewed articles on the relationship between organic egg consumption and human health issues indexed from the inception date to 13 April 2022. No date or language limits will be established. The search syntax included the terms eggs, organic or ecological and human and possible variations combined by means of Boolean operators appropriate to each base. The detailed search strategy is found in the **Supplementary Material**. In addition, after copying the syntax in the Google Academic search engine, the first twenty pages were observed in search of studies of interest that had not been found in the main databases. With the same objective, the reference lists of the reviews found on the subject were also examined.

The following inclusion criteria were defined according to the *PICOS* structure: a) population: people of all ages; b) intervention/exposure: consumption of organic or ecological eggs; c) comparison: consumption of non-organic eggs, regardless of the type of production method, or no consumption of eggs; d) outcome: any chemical, physical or psychological parameter related to human health; e) study design: cross-sectional or follow-up observational studies or clinical trials. Exclusion criteria were established for not submitting results of the association of interest, when duplicate reports of the same study were submitted or included an ineligible publication format, such as event abstracts, preprints and literature reviews.

After excluding the duplicate studies identified in the different databases, the titles and abstracts were reviewed to rule out those clearly outside the intended scope. Of the remaining studies, the full text was examined to confirm whether the inclusion criteria were met. The data of interest were extracted from the studies finally included. It was not necessary to contact any author to request data not available in the article. Two independent reviewers (AEM and RF-R) extracted the following information from the studies selected for inclusion: author(s) and year; country, design, follow-up, washout, response rate, characteristics of the population, sample size, context, mean age of the participants, women percentage, exposure variable, outcome measures, and main results. A third coauthor (JFL-G) was consulted when disagreements between the initial reviewers occurred.

For the assessment of the risk of bias of each study, the NIH Quality Assessment Tool for Observational Cohort and Cross-sectional Studies (22) was applied, as well as the Rob2 (23) from the Cochrane for the randomized controlled trials. The NIH Quality Assessment Tool for Observational Cohorts and Cross-Sectional Studies was used to assess the risk of bias in cohort and cross-sectional studies. Accordingly, 14 items determined the risk of systematic bias based on clarity of the research question, participation rate, follow up and drop-outs, power analysis, exposure, and outcome time of measurement. The items are scored as “yes,” “no,” “not reported,” “cannot be determined” or “not applicable.” Depending on this, each study was classified as “poor,” “fair” or “good.” Cochrane’s Risk-of-Bias 2 (RoB 2) tool was used to assess the risk of bias of the included RCTs, in which the following domains were assessed: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. Then, each of the five domains was graded individually as “low risk of bias,” “some concerns” or “high risk of bias” 0.26 Finally, each study was classified for the overall risk of bias as (1) “low risk of bias” when a low risk of bias was determined for all domains; (2) “some concerns” when at least one domain was assessed as raising some concerns but not to be at high risk of bias for any single domain; or (3) “high risk of bias” when a high risk of bias was reached for at least one domain or some concerns in multiple domains.

The included studies were analyzed separately, and a synthesis of their main characteristics and results was developed.

RESULTS

Out of a total of 3,419 articles identified in the search, 3330 were discarded due to the title and abstract. Of the remaining 89, reading the full text resulted in the exclusion of 87 studies for the reasons specified in **Figure 1**. Three studies (10, 11, 24) were finally included in this systematic review.

Table 1 presents the main characteristics and results of the included studies. The oldest study (2008) was conducted in

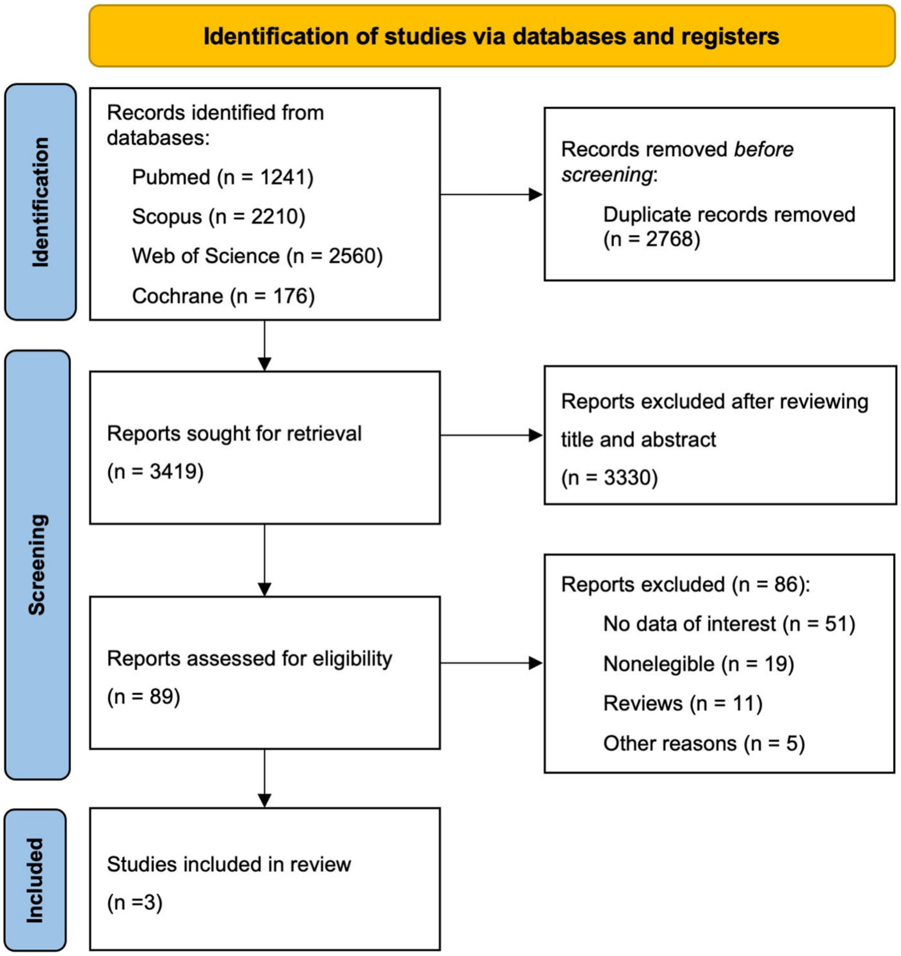

FIGURE 1 | PRISMA 2020 flow diagram of study selection.

The Netherlands (10), and the next two were conducted in the United States (11, 24). Each study had a different type of design. In the first study, a cohort of 2,583 newborns was followed up to 2 years of age with the aim of analyzing whether the consumption of organic foods, including eggs, moderate (from 50 to 90% of consumption occasions) or strict (more than 90% of the time) was associated with an increased likelihood of developing eczema compared with eating conventional eggs (i.e., less than half of the time choosing organic eggs) (10). Although the crude analyses indicated unfavorable results for the consumption of organic eggs (i.e., greater probability of having eczema), when controlling for the confounding effect of other exposures, such as breast-feeding, pet, day-care, tobacco, etc., that detrimental effect was not sustained.

The second study, conducted in 2012, was a randomized crossover clinical trial including 20 free-living lacto-ovo-vegetarian adults with the aim of analyzing the transfer of 3 types of carotenoids from eggs to blood serum (24). In the intervention phase, the participants received 6 organic eggs per week for 8 weeks, and with a washout time of 4 weeks, the control phase was carried out, in which they did not consume eggs. The final results revealed an increase in beta-carotene lutein after eating organic eggs compared to the period without eating eggs.

On the other hand, the third study was a cross-sectional design in which 3,815 adults over 50 years of age were questioned about the consumption or non-consumption of organic eggs, and they had blood samples collected to assess the levels of inflammatory markers (11). In the analysis adjusted for sociodemographic and lifestyle confounders, including caloric intake, organic egg consumers had slightly lower levels of C-reactive protein (CRP) and cystatin C (CysC) than those consuming conventional eggs (11) (**Table 1**).

Finally, the risk of bias assessment of the included studies revealed that among observational studies, one scored as "Good" (10) and the other as "Fair" (11) quality according to the NIH tool. The randomized controlled trial had an overall risk of bias of some concerns (24). The detailed risk of bias assessment of each study is found in the **Supplementary material** (**Table S1** and **Figure S1**).

TABLE 1 | Characteristics of the included studies.

Author, year	Study Country Design Follow-up Washout Response rate	Population Sample size Context Mean age % Female	Exposure variable	Dependent variables	Main results
Burns-Whitmore et al. (24)	United States Randomized crossover trial 8 weeks 4 weeks 76.9%	20 Lacto-ovo-vegetarian adults 38 ± 3 years 80.0%	Six organic eggs/week (intervention) *vs.* no eggs (control)	Serum carotenoids: Lutein ß-carotene Zeaxanthin	Compared with the control, in organic egg treatment lutein was significantly higher ($p = 0.009$), ß-carotene increased only approached significance ($p = 0.066$) and zeaxanthin was not associated ($p = 0.139$).
Kummeling et al. (10)	The Netherlands Cohort NA NA 94.5%	2583 children 2 years 49.0%	Moderate (50–90%) and strictly (>90%) organic egg *vs.* conventional (<50%) egg consumption	Eczema	In analysis adjusted for sociodemographic and several other exposures (breast-feeding, pet, day-care, tobacco, etc.), neither moderate (OR: 1.40; 95% CI: 0.98, 1.99) nor strictly (OR: 1.03; 95% CI: 0.76, 1.38) organic egg consumption was associated with eczema.
Ludwig-Borycz et al. (11)	United States Cross-sectional NA NA 47.3%	3815 Free-living >50 years 63.5 ± 14.6 years 57.3%	Organic eggs *vs.* no organic eggs	C-reactive protein Cystatin C	In analysis adjusted for sociodemographic and lifestyle confounders including caloric intake, organic egg consumers had lower CRP (log: −0.091; 95% CI: −0.181, −0.001) and CysC (log: −0.046; 95% CI: −0.071, – 0.022).

CI, confidence interval; CRP, C-reactive protein; CysC, Cystatin C; NA, Not applicable; OR, odds ratio.

DISCUSSION

This systematic review is pioneering in studying the relationship between organic egg consumption and aspects related to human health. To date, this topic has been explored in only three studies with different designs and population groups studied. Although two of the studies reported favorable results in terms of higher serum carotenoid levels (24) and lower levels of specific inflammatory markers (11) associated with the consumption of organic eggs, this limited set of results does not yet allow firm conclusions to be drawn about the benefits of organic eggs on human health.

Egg allergy is the second most common food allergy in infants and young children after cow's milk allergy and can pose quality of life concerns (25). In addition to avoiding egg consumption, it has been reported that the introduction of eggs in the diet between 3 and 6 months of life could reduce the risk of egg allergy (26). With the expansion of organic agriculture and its possible lower allergenic potential, justified by the higher concentration of nutrients with anti-inflammatory potential such as omega-3 polyunsaturated fatty acids (20), it has been suggested that the intake of organic eggs could also represent a dietary alternative for children allergic or predisposed to egg allergy. Although the KOALA Birth Cohort Study conducted in The Netherlands found no prospective association between moderate or strict consumption of organic eggs (as well as organic meat, fruits, and vegetables) in infants from birth to 2 years of age, the authors reported that the consumption of organic dairy products was associated with a lower risk of eczema (10). In quantitative terms, exposure to egg proteins is expected to be lower than exposure to milk throughout this stage of life. For this reason, the advancement of knowledge about the allergenic potential of organic vs. conventional eggs still depends on longer-term prospective studies with repeated measurements of the consumption of eggs and other organic foods.

In the randomized crossover clinical trial conducted by Burns-Whitmore et al. (24), a higher concentration of lutein in the blood of lacto-ovo-vegetarian adults was observed when they consumed approximately one organic egg daily compared to when they did not consume eggs (24). Elevated levels of this carotenoid in the blood may play an important role in the prevention of macular degeneration and age-related vision loss (27). Although, according to the authors, organic eggs provide a bioavailable source of lutein (a carotenoid with anti-inflammatory properties), it is necessary to consider that the control group did not consume eggs of any kind. For this reason, it could not be affirmed that the contribution of carotenoids was higher when organic eggs were consumed instead of conventional eggs.

Regarding the third and the most recent scientific evidence on the subject, in the nationally representative, longitudinal panel study of Americans over 50, significantly lower levels of two important inflammatory markers were found among individuals who consumed organic eggs compared to those who consumed conventional or non-organic eggs. It is noteworthy that these results were observed independently of several important confounders, such as body mass index, blood pressure, diabetes, physical activity, and total daily caloric intake. Considering the accumulation of evidence on the inflammatory potential of the diet in cardiometabolic risk (28), the aforementioned study sheds light on the possible additive anti-inflammatory effect of organic vs. non-organic feeding (24). However, these findings certainly need to be confirmed in future prospective studies and, furthermore, replicated in populations with dietary patterns different from the Westernized, predominant in the country studied, the United States.

Our results indicate that scientific evidence has thus far not focused on whether organic eggs are directly associated with health benefits. What has indeed advanced—not only for eggs but also for other organically produced foods—was research on the nutritional value of organic foods compared to conventional foods (6), which in turn could lead to advantages for human health (7). For instance, organic vegetables have fewer environmental pollutants (29), which are related to cardiovascular risk (30) and cancer (31). In addition, organic foods of animal origin provide a better lipid profile, vitamins and minerals involved in the etiology of many diseases throughout the life cycle, as well as lower levels of contamination by microorganisms related to gastrointestinal disorders (7, 32).

Specifically, it has been found in studies based on biochemical analyses of egg composition that, compared to conventional eggs, organic eggs are lower in saturated fat (conventional eggs: 85.7 g kg−1 yolk; organic eggs: 68.1 g kg−1 yolk) (20), have a lower n-6/n-3 ratio (conventional eggs: 11.5; organic eggs: 7.8) (20), and have fewer endocrine disruptors such as dimethyl phthalate (DMP) (conventional eggs: 76%; organic eggs: 52%) (33). In addition, other review studies reported that organic eggs are less exposed to antibiotics (34) and are less contaminated by Salmonella (35). These biological differences are supposed to be largely explained by animal feed based on feed made with mostly organic products and under extremely hygienic conditions (29, 36). In particular, on the diet of the hens in different types of creation, compared to the feed commonly used in conventional cage creation, organic farming (and in cage-free farming) favors the predominant consumption of grass from external areas, which provides high amounts of fiber, tocopherol, carotenoids and flavonoids (20), allowing a remarkable transfer of bioactive substances that could confer higher nutritional quality to the organic egg compared to the conventional egg. In addition, it has also been shown that cage-free housing and the guarantee of adequate outdoor space for the animal behavior of all livestock ensures that laying hens can move freely during some hours of the day and, consequently, are less stressed (37). It is understandable that the result of this set of measures based on sustainability, high-quality animal nutrition, and animal welfare results in a feed that generates a lower environmental footprint (38, 39) and, above all, a higher nutritional density (6) compared to conventional egg production. It is also reasonable to consider that future clinical and epidemiological studies could test the hypothesis of whether these better nutritional qualities translate into concrete benefits for people who consume eggs from organic farms.

The main limitation of this review is the small number and heterogeneous design of the included studies, which limits the conclusions that can be drawn and does not justify recommendations on the consumption of organic eggs. In particular, it is noteworthy that the three studies used different comparison groups to analyze the potential benefits of organic egg consumption, which prevents comparability between them. In the two observational studies, the comparison group was composed of individuals who consumed organic eggs less than 50% of the time (10) or did not consume organic eggs (11), while the experimental study considered not consuming eggs of any type as the comparison group (24). The scarcity and methodological heterogeneity of studies are inherent limitations at the early stage of research on any organic food. The main reasons why no progress has yet been made in understanding whether adherence to a dietary pattern based on organic food translates into benefits for human health are that consumption is still very low and strictly related to socioeconomic status (40) and, consequently, to better health status.

In conclusion, preliminary evidence from human studies suggests that organic eggs may have nutritional advantages over conventional or non-organic eggs, possibly related to the higher levels of carotenoids and the reduction in the inflammatory potential of the diet. We advocate the inclusion of questions about the frequency of consumption of organic foods in future population-based dietary surveys. In addition, on the one hand, it is important to defend that laboratory studies are essential to discover biological differences related to the composition of organic foods and have thus far presented promising results in their favor. Last, knowledge about the real impact of organic agricultural production, and in particular of organic eggs, on human health requires clinical trials with samples, follow-up time and control of several confounding variables specifically designed for that purpose.

AUTHOR CONTRIBUTIONS

VM-V, AM, and MG-M conceived the present idea. AM and MG-M performed the literature searches and data extraction. RF-R and BB-P assessed the risk of bias of the included studies. AM drafted the first manuscript version. All authors provided intellectual content and approved the final manuscript version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Hansmann R, Baur I, Binder CR. Increasing organic food consumption: an integrating model of drivers and barriers. *J Clean Prod.* (2020) 275:123058. doi: 10.1016/j.jclepro.2020.123058
2. Thøgersen J. Country differences in sustainable consumption: the case of organic food. *JMK.* (2010) 30:171–85. doi: 10.1177/0276146 710361926
3. Goetze F, Ferjani A, Götze F, Ferjani A. Who buys organic foods in Switzerland? *Agrarforschung Schweiz.* (2014) 5:338–43.
4. Joo D-N, Li C, Khan A, Qalati S, Naz S, Rana F. Purchase intention toward organic food among young consumers using theory of planned behavior: role of environmental concerns and environmental awareness. *J Environ Plan. Manag.* (2020) 64:796–822. doi: 10.1080/09640568.2020.1785404
5. Ashraf M. What drives and mediates organic food purchase intention: an analysis using bounded rationality theory. *J Int Food Agribus. Mark.* (2020) 33:1–32. doi: 10.1080/08974438.2020.1770660
6. Mie A, Andersen HR, Gunnarsson S, Kahl J, Kesse-Guyot E, Rembiałkowska E, et al. Human health implications of organic food and organic agriculture: a comprehensive review. *Environ Health.* (2017) 16:111. doi: 10.1186/s12940-017-0315-4
7. Brantsæter AL, Ydersbond TA, Hoppin JA, Haugen M, Meltzer HM. Organic food in the diet: exposure and health implications. *Annu Rev Public Health.* (2017) 38:295–313. doi: 10.1146/annurev-publhealth-031816-044437
8. Kamalakannan S, Mathieu IP. Organic food: nutritional and environmental considerations. *Pediatr Rev.* (2021) 42:345–7. doi: 10.1542/pir.2020-002980
9. Dangour AD, Lock K, Hayter A, Aikenhead A, Allen E, Uauy R. Nutrition-Related health effects of organic foods: a systematic review. *Am J Clin Nutr.* (2010) 92:203–10. doi: 10.3945/ajcn.2010.29269
10. Kummeling I, Thijs C, Huber M, van de Vijver LPL, Snijders BEP, Penders J, et al. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. *Br J Nutr.* (2008) 99:598–605. doi: 10.1017/S0007114507815844
11. Ludwig-Borycz E, Guyer HM, Aljahdali AA, Baylin A. Organic food consumption is associated with inflammatory biomarkers among older adults. *Public Health Nutr.* (2021) 24:4603–13. doi: 10.1017/S13689 80020005236
12. Miranda JM, Anton X, Redondo-Valbuena C, Roca-Saavedra P, Rodriguez JA, Lamas A, et al. Egg and egg-derived foods: effects on human health and use as functional foods. *Nutrients.* (2015) 7:706–29. doi: 10.3390/nu7010706
13. Réhault-Godbert S, Guyot N, Nys Y. The golden egg: nutritional value, bioactivities, and emerging benefits for human health. *Nutrients.* (2019) 11:684. doi: 10.3390/nu11030684
14. Campmajó G, Núñez O. Authentication of Conventional and Organic Eggs. In: *Chromatographic and Related Separation Techniques in Food Integrity and Authenticity*. Munich: World Scientific Publishing Co Pte Ltd. p. 187–213.
15. Alagawany M, Abd El-Hack ME, Farag MR. *Nutritional Strategies to Produce Organic and Healthy Poultry Products*. Cham: Springer International Publishing (2019). p. 339–56.
16. United States Department of Agriculture. USDA Organic. *Guidelines for Organic Certification of Poultry*. (2022). Available online at: https://www.ams.usda.gov/rules-regulations/organic (Accessed April 23, 2022).
17. Banaszewska D, Biesiada-Drzazga B, Marciniuk M, Hrnčár C, Arpášová H, Kaim-Mirowski S, et al. Comparison of the quality of cage and organic eggs available in retail and their content of selected macro-elements. *Acta Sci Pol Technol Aliment.* (2020) 19:159–67. doi: 10.17306/J.AFS.2020.0797
18. Marelli SP, Madeddu M, Mangiagalli MG, Cerolini S, Zaniboni L. Egg production systems, open space allowance and their effects on physical parameters and fatty acid profile in commercial eggs. *Animals.* (2021) 11:265. doi: 10.3390/ani11020265
19. Methner U, Diller R, Reiche R, Böhland K, Boehland K. Occurence of salmonellae in laying hens in different housing systems and conclusion for the control. *Berl Munch Tierarztl Wochenschr.* (2006) 119:467–73.
20. Mugnai C, Sossidou EN, Dal Bosco A, Ruggeri S, Mattioli S, Castellini C. The effects of husbandry system on the grass intake and egg nutritive characteristics of laying hens. *J Sci Food Agric.* (2014) 94:459–67. doi: 10.1002/jsfa.6269
21. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The Prisma 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* (2021) 372:n71. doi: 10.1136/bmj.n71
22. National Institutes of Health. *Quality Assessment Tool for Observational Cohort and Cross Sectional Studies.* (2014). Available online at: https://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools/cohort (a=ccessed 23 April, 2022).
23. Sterne JAC, Savovic J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. Rob 2: a revised tool for assessing risk of bias in randomised trials. *BMJ.* (2019) 366:l4898. doi: 10.1136/bmj.l4898
24. Burns-Whitmore BL, Haddad EH, Sabaté J, Jaceldo-Siegl K, Tanzman J, Rajaram S, et al. Effect of N-3 fatty acid enriched eggs and organic eggs on serum lutein in free-living lacto-ovo vegetarians. *Eur J Clin Nutr.* (2010) 64:1332–7. doi: 10.1038/ejcn.2010.140
25. Caubet JC, Wang J. Current understanding of egg allergy. *Pediatr Clin North Am.* (2011) 58:427–43, xi. Epub 2011/04/02. doi: 10.1016/j.pcl.2011.02.014
26. Al-Saud B, Sigurdardottir ST. Early introduction of egg and the development of egg allergy in children: a systematic review and meta-analysis. *Int Arch Allergy Immunol.* (2018) 177:350–9. doi: 10.1159/000492131
27. Nolan JM, Stack J, O'Connell E, Beatty S. The relationships between macular pigment optical density and its constituent carotenoids in diet and serum. *Invest Ophthalmol Vis Sci.* (2007) 48:571–82. doi: 10.1167/iovs.06-0864
28. Hariharan R, Odjidja EN, Scott D, Shivappa N, Hebert JR, Hodge A, et al. The Dietary inflammatory index, obesity, type 2 diabetes, and cardiovascular risk factors and diseases. *Obes Rev.* (2022) 23:e13349. doi: 10.1111/obr.13349
29. Ramakrishnan B, Maddela NR, Venkateswarlu K, Megharaj M. Organic farming: does it contribute to contaminant-free produce and ensure food safety? *Sci Total Environ.* (2021) 769:145079. doi: 10.1016/j.scitotenv.2021.145079
30. O'Toole TE, Conklin DJ, Bhatnagar A. Environmental risk factors for heart disease. *Rev Environ Health.* (2008) 23:167–202. doi: 10.1515/reveh.2008.23.3.167
31. Vogt R, Bennett D, Cassady D, Frost J, Ritz B, Hertz-Picciotto I. Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: a risk assessment. *Environ Health.* (2012) 11:83. doi: 10.1186/1476-069X-11-83
32. Lairon D. Nutritional quality and safety of organic food. *Agron Sustain Dev.* (2010) 30:33–41. doi: 10.1051/agro/2009019
33. Kuzukiran O, Yurdakok-Dikmen B, Sevin S, Sireli UT, Iplikcioglu-Cil G, Filazi A. Determination of selected endocrine disruptors in organic, free-range, and battery-produced hen eggs and risk assessment. *Environ Sci Pollut Res Int.* (2018) 25:35376–86. doi: 10.1007/s11356-018-3400-5
34. Abd El-Hack ME, El-Saadony MT, Salem HM, El-Tahan AM, Soliman MM, Youssef GBA, et al. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poultry Sci.* (2022) 101:101696. doi: 10.1016/j.psj.2022.101696
35. Sosnowski M, Osek J. Microbiological safety of food of animal origin from organic farms. *J Vet Res.* (2021) 65:87–92. doi: 10.2478/jvetres-2021-0015
36. Williams CM. Nutritional quality of organic food: shades of grey or shades of green? *Proc Nutr Soc.* (2002) 61:19–24. doi: 10.1079/pns2001126
37. Bryden WL Li X, Ruhnke I, Zhang D, Shini S. Nutrition, feeding and laying hen welfare. *Animal Production Sci.* (2021) 61:893–914. doi: 10.1071/AN20396
38. Anderson KE. Overview of natural and organic egg production: looking back to the future1 1papers from the current and future prospects for natural and organic poultry symposium were presented at the poultry

science association's 97th annual meeting in Niagara Falls, Ontario, Canada. *J Applied Poul Res.* (2009) 18:348–54. doi: 10.3382/japr.2008-00119

39. Cattell Noll L, Leach AM, Seufert V, Galloway JN, Atwell B, Erisman JW, et al. The nitrogen footprint of organic food in the United States. *Environ Res Letters.* (2020) 15:045004. doi: 10.1088/1748-9326/ab7029
40. Strzok JL, Huffman WE. Willingness to pay for organic food products and organic purity: experimental evidence. *AgBioForum.* (2015) 18:345–53.

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Association of Household Food Insecurity With Dietary Intakes and Nutrition-Related Knowledge, Attitudes, and Practices Among School-Aged Children in Gaza Strip, Palestine

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Background: The present study aimed to determine the association of household food insecurity with dietary intakes and nutrition-related knowledge, attitudes, and practices (KAP) among school-aged children.

Methods: This cross-sectional study was conducted among a representative sample of school-aged children. A total of 380 children and their parents were selected from all Gaza strip governorates, using a random sampling method. The demographic and socioeconomic characteristics; the Radimer/Cornell food security scale; two non-consecutive days of 24-h dietary recall; anthropometric measurements; and the Food and Agriculture Organization KAP-questionnaire (Module 3) were employed. Statistical analysis was performed using SPSS version 25.

Results: About 71.6% of school-aged children were household food-insecure, while 28.4% were household food-secure. Significant associations were found between living area, educational level, household monthly income, weight for age and BMI for age z-scores, underweight, malnutrition status, intakes of protein, iron, vitamin D, and zinc among household food-secure, and household food-insecure. After adjustment for confounding variables, having nutrition-related adequate KAP were associated with lower odds of being food-insecure household [$OR = 0.519$, 95% ($CI = 0.320–0.841$)], [$OR = 0.510$, 95% $CI = (0.315–0.827)$], and [$OR = 0.466$, 95% $CI = (0.285–0.763)$, $P < 0.05$ for all], respectively.

Conclusions: Low socioeconomic status, low anthropometric indices, poor dietary intakes may be associated with a high level of food-insecurity; while having nutrition-related adequate KAP may be protective against food-insecurity among school-aged children.

Keywords: attitudes, dietary intakes, food insecurity, nutrition-related knowledge, practices, school-aged children

INTRODUCTION

Food insecurity is described as a scenario in which people do not always have access to enough, safe, and nutritious food to satisfy their dietary demands for an active and healthy life while also taking their food preferences into account (1, 2). Worldwide, the number of people affected by moderate or severe food insecurity will continue to increase in 2022. In addition, nearly one in three people worldwide did not have access to adequate food, with an increase of almost 320 million people in just 1 year (3). The high cost of healthy diets coupled with persistently high levels of income inequality put healthy diets out of reach for around three billion people, especially the poor, in every region of the world (4). Most of these people live in Asia (1.85 billion) and Africa (1.0 billion) (5).

Despite international and national efforts to eradicate severe poverty and enhance the global food supply, food insecurity continues to be a significant problem that affects people all over the world, especially those in low- and middle-income nations (6). According to recent research, food insecurity is concentrated mainly in conflict-affected countries experiencing political unrest, financial instability, and relocation (7, 8). Furthermore, the Palestinian situation was one in which war remained the primary cause of food insecurity, affecting the lives of two million of the population and exacerbated by high poverty and unemployment rates (9). Additionally, protracted conflict, economic stagnation and restricted trade and access to resources, coupled with high unemployment poverty rates, continue to pose serious challenges to the achievement of food security and improved nutrition (10). Nearly seven out of ten people in Gaza are impoverished, half of the workforce is jobless, and seven out of ten families are food insecure (11). Moreover, based on recent data, over 68 percent of households in the Gaza strip are food-insecure (12).

Food insecurity has the potential to be harmful to individuals of any age, but it can be especially devastating to school-aged children (13). School-aged children who experience food insecurity may suffer from low cognitive abilities poor academic performance, emotional, behavioral, mental, and physical consequences (14). Many of the previous studies show that food-insecure school-aged children are almost twice as likely to be in poor health when compared to food-secure children and are significantly more likely to be hospitalized (15, 16). On the other hand, nutrition-related knowledge, attitudes and practices (KAP) are necessary for dietary changes toward a healthier dietary pattern (17). For that reason, food and nutrition-related KAP is one of the key factors to achieving households food and nutritional security (18). In fact, earlier research has focused on young children (under the age of 5 years) (19, 20), with older children receiving less attention (16). Recent research suggests that school-aged children are just as exposed, if not more prone, to the negative repercussions of household food insecurity than their younger siblings (21, 22). Due to their reliance on the table food available in the home, school-aged children may be at an increased risk of health problems associated with food insecurity compared to their younger siblings, who may still be nutritionally protected by breastfeeding and other early infant feeding practices (23). Furthermore, school-aged children have more critical dietary requirements. However, compared to their younger children, moms may be less aware of their nutritional intakes or have less influence over their older children's food and beverage choices outside the house (24). Therefore, the present study was conducted to determine the association of household food insecurity with dietary intakes and nutrition-related KAP among school-aged children in the Gaza Strip, Palestine.

MATERIALS AND METHODS

Study Design

This cross-sectional study was conducted in the year 2021 among a representative sample of school-aged children aged more than 5 to <18 years. A total of 380 school-aged children and their parents were selected from all Gaza strip governorates based on the population density, using a random sampling method.

Eligibility Criteria

Households having at least one school-aged child (male or female), aged more than 5 to <18 years, and living with his/her mother in the same household, and mothers and fathers aged ≥ 18 years and having school-aged children were included in the present study. On the contrary, households without school-aged children, mothers and fathers with disabilities or chronic disease, and school-aged children with disabilities or chronic disease were excluded from the present study.

Study Location

The current study was conducted in the households of the Gaza strip, Palestine. The estimated population of the Gaza strip is about 2,106,745 million. Gaza strip is divided into five governorates: North-Gaza, Gaza, Middle-Area, Khanyounis, and Rafah, with a population density of 19.3, 34.9, 14.4, 19.1, and 12.2%, respectively (25).

Sample Size and Sampling

In the present study, the representative sample size was calculated using the following formula.

$$Sample\ size\ (n) = \frac{Z_{1-\alpha/2}^{2}\ P(1-P)}{d^{2}} = \frac{(1.96)^{2}\ (0.50)\ (1-0.50)}{(0.05)^{2}} = 380 \quad (1)$$

Where, $Z_{1-\alpha/2}$ = Standard normal variate (Z value is 1.96 for a 95 percent confidence level); p = Response distribution (50%); and d = Margin of error (5%).

Accordingly, 380 school-aged children and their parents were recruited in this study, applying a random sampling method. The sample was distributed into the five governorates of the Gaza strip based on the population density as follows: 73 from North-Gaza, 133 from Gaza, 55 from Middle-Area, 73 from Khanyounis, and 46 from Rafah.

Tools of the Study

Interview Questionnaire

To achieve the purpose of the study, an interview-based questionnaire was used; the data was collected from the school-aged children and their parents (mothers and fathers) by ten qualified data collectors who were given a full explanation and training by the researcher about the study. The data collectors went to the participants' homes, and the study participants did not go to any research center. The questionnaire contains items about the demographic and socioeconomic characteristics of the school-aged children; the 10-item Radimer/Cornell food security scale to assess the household food security status (26); two non-consecutive days of 24-h dietary recall for dietary intakes assessment; anthropometric measurements; and the Food and Agriculture Organization (FAO) of the United Nations questionnaire (Module 3: Diet of school-aged children) to assess the nutrition-related KAP of school-aged children (27). Before data collection, a pilot study was carried out on 30 participants to enable the researcher to examine the tools of the study. The questionnaire and data collection process were adjusted according to the result of the pilot study. In addition, during pilot study, test-retest reliability was undertaken, where the same tool was administered to the same participants twice and the results were compared for congruency.

Demographic and Socioeconomic Characteristics of School-Aged Children

An individual face-to-face interview was conducted with the school-aged children and their parents (mothers and fathers) to collect information about demographic and socioeconomic characteristics of school-aged children, including age (years), gender, governorate, the nature of the living area, educational level of the head of households (mothers or fathers), and monthly household income (NIS). The used categories of educational level and monthly household income (NIS) variables in the current study were similar to which mentioned in earlier studies in Gaza strip (28, 29).

Assessment of Households' Food Security Status

The 10-items Radimer/Cornell food security scale was used for determining the households' food security status. The scale is a valid and reliable tool for measuring household food insecurity in a culturally diverse setting (30), including Palestine (28, 29). An interview was conducted with the head of households (mothers or fathers) to collect data about the households' food security status. Then, the households were classified by food security status as follow: (1) Household food secure: Negative answers to all hunger and food insecurity items; (2) Household food insecurity: Positive answers ("sometimes true" or "often true") to one or more hunger and food insecurity items.

Dietary Intakes Assessment of School-Aged Children

Two non-consecutive days of 24-h dietary recall were employed to determine the quantity of macro-and micronutrients consumed by the school-aged children. The school-aged children and their parents (mothers and fathers) were requested to recall all beverages and food consumed by the school-aged children in the past 24 h. In the present study, the data were collected on the first day of the two non-consecutive days of 24-h dietary recall by a face-to-face interview, and then the data collectors obtained each participant's phone number and called him/her later to obtain data about another different day. The portion sizes were estimated using a set of household measurements (i.e., plates, cups, glasses, and spoons). Dietary data from the 24-h dietary recall was processed by hand (office work) in order to calculate the net grams of foods consumed by school-aged children. This information was analyzed using the Nutritionist Pro Software version 7.1.0 (Axxya Systems, USA) (31) to determine energy (kcal) and nutrients intakes, including protein (g), carbohydrate (g), fat (g), iron (mg), vitamin A (μg), vitamin D (μg), calcium (mg), and zinc (mg). Additionally, the nutritional calculations were performed based on the USDA Food Composition Database.

Anthropometric Measurements of School-Aged Children

Height and Weight

The height (cm) and weight (kg) of the school-aged children were measured following standard recommended procedures. A digital weighing scale (to the nearest 0.1 kg) (SECA, Germany) and a stadiometer (with the precision of 0.1 cm) were used (32). All measurements were taken twice, and the average of the two values was reported. The body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (33). Furthermore, the age, weight, height, and BMI of the children were translated into three indices: weight for age (WAZ), height for age (HAZ), and BMI for age (BMIZ), which were expressed in terms of z-scores using the WHO Anthro Software (Version 3, 2009) (34). The school-aged children were classified into moderate and severe underweight and moderate and severe stunting, which mean that WAZ and HAZ z-scores are < -2 and < -3, respectively (35). Then, the school-aged children were classified based on their malnutrition status into obesity, overweight, thinness, and severe thinness, which mean that BMIZ is $> +2$, $> +1$, < -2, and < -3 SD, respectively (36).

TABLE 1 | Demographic and socioeconomic characteristics of school-aged children by food security status.

Variables	Household food-secure (n = 108)	Household food-insecure (n = 272)	P-value
Age (years)			
Mean ± SD	10.85 ± 3.57	10.82 ± 3.58	0.952[a]
Gender			
Males	60.0 (55.6)	155 (57.0)	0.800[b]
Females	48.0 (44.4)	117 (43.0)	
Governorate			
North Gaza	15.0 (13.9)	58.0 (21.3)	0.063[b]
Gaza	35.0 (32.4)	97.0 (35.7)	
Middle Area	22.0 (20.3)	32.0 (11.8)	
Khanyounis	18.0 (16.7)	55.0 (20.2)	
Rafah	18.0 (16.7)	30.0 (11.0)	
Living area			
City	39.0 (36.2)	103 (37.9)	**0.036[b]***
Village	9.0 (8.3)	38.0 (14.0)	
Camp	60.0 (55.5)	131.0 (48.1)	
Educational level of the head of households (mothers or fathers)			
Low education	42.0 (38.8)	144 (52.9)	**0.049[b]***
High education	66.0 (61.2)	128 (47.1)	
Household monthly income (NIS)			
≤ 2,000	48.0 (44.4)	164 (60.3)	**0.004[b]***
> 2,000	60.0 (55.6)	108 (39.7)	

[a]*Independent Samples t-test.*
[b]*Chi Square Test.*
[*]*Difference is significant at the 0.05 level (two tailed).*
NIS, New Israeli Shekel.
Difference is significant at the 0.05 level (two tailed).

Mid-upper Arm Circumference

Mid-upper Arm Circumference (MUAC) (cm) was recorded to the nearest 0.1 cm using the MUAC measuring tape. Investigators were measured the MUAC at the midpoint of the arm, where the measuring tape was snugged to the skin but not pressing soft tissues (37). All measurements were taken twice, and the average of the two values was reported.

Assessment of Nutrition-Related KAP

The FAO of the United Nations questionnaire (Module 3: Diet of school-aged children) for assessing nutrition-related KAP of school-aged children was used to conduct high-quality surveys (17). Accordingly, we have used the same valid questionnaire distributed by the FAO in Palestine in 2017 (38). The questionnaire comprises predefined questions that capture information on critical KAP related to the diet of school-aged children. In the present study, the nutrition-related KAP consists of two questions related to nutrition-related knowledge, six questions related to nutrition-related attitudes, and 12 questions related to nutrition-related practices (39). Additionally, data regarding the nutrition-related KAP of school-aged children were collected from the head of households (mothers or fathers) using an interview-based questionnaire.

Data Analysis

The Statistical Package for Social Science (SPSS) for Windows (version 25) was used for data analysis. Descriptive statistics were used to describe continuous and categorical variables. The chi-square test and fisher's exact test were used to determine the significant differences between categorical variables. The differences between mean were tested by independent samples t-test. Furthermore, crude and adjusted odds ratio (OR) and 95% confidence interval (CI) for the overall nutrition related-KAP of school-age children by household food-security status were calculated using binary logistic regression. A $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 presents the demographic and socioeconomic characteristics of school-aged children by food security status. A total of 380 school-aged children (43.4 females and 56.6% males) were included in the current study. About 272 (71.6%) of the included households were food insecure, while only 108 (28.4%) were food secure. Nearly half of the food-insecure households, 131 (48.2%), were located at refugee camps. About 144 (52.9%) of the head of households (mothers or fathers) who belong to food-insecure households had a low education level. A large percentage of food-insecure households, 164 (60.3%),

TABLE 2 | Prevalence of undernutrition and anthropometric parameters of school-aged children by food security status.

Measurements	Household food-secure (*n* = 108)	Household food-insecure (*n* = 272)	*P*-value
Weight (kg)			
Mean ± SD	39.98 ± 17.01	33.40 ± 16.24	**0.041**[a*]
Height (cm)			
Mean ± SD	137.66 ± 19.04	136.76 ± 20.58	0.697[a]
MUAC (cm)			
Mean ± SD	22.52 ± 5.86	21.57 ± 4.78	0.102[a]
WAZ (z-score)			
Mean ± SD	0.75 ± 1.05	−0.37 ± 1.20	**< 0.001**[a*]
HAZ (z-score)			
Mean ± SD	−0.74 ± 1.26	−0.79 ± 1.51	0.760[a]
BMIZ (z-score)			
Mean ± SD	1.94 ± 0.57	−0.18 ± 1.09	**< 0.001**[a*]
Underweight (weight for age)			
Normal	95.0 (88.0)	25.0 (9.2)	**0.001**[b*]
Moderate	10.0 (9.3)	227 (83.5)	
Severe	3.0 (2.7)	20.0 (7.3)	
Stunting			
Normal	91.0 (84.2)	214 (78.7)	0.390[b]
Moderate	11.0 (10.2)	38.0 (14.0)	
Severe	6.0 (5.6)	20.0 (7.3)	
Malnutrition status			
Obesity	0.0 (0.0)	0.0 (0.0)	**< 0.001**[c*]
Overweight	9.0 (8.3)	0.0 (0.0)	
Normal nutritional status	94.0 (87.0)	164 (60.3)	
Thinness	5.0 (4.7)	98.0 (36.0)	
Severe thinness	0.0 (0.0)	10.0 (3.7)	

MUAC, Mid upper arm circumference; WA, weight for age z-score; HAZ, Height for age z-score; BMIZ, Body Mass Index for age z-score.
Moderate and severe underweight, and moderate and severe stunting mean that weight for age, and height for age z-scores are < −2 and < −3, respectively (34).
Obesity, overweight, thinness, and severe thinness mean that BMIZ is > +2, > +1, < −2, and < −3 SD, respectively (35).
[a]*Independent Samples t-test.*
[b]*Chi square test.*
[c]*Fisher's Exact Test.*
[*]*Difference is significant at the 0.05 level (two tailed).*
Difference is significant at the 0.05 level (two tailed).

had monthly household income ≤2,000 New Israeli Shekel (NIS)—the local currency. There were statistically significant association differences between household food-secured and household food-insecured regarding the nature of the living area, educational level of school-aged children, and monthly household income ($P = 0.036$, 0.049, and 0.004, respectively).

In addition, **Table 2** shows the anthropometric parameters and status of undernutrition among school-aged children by food security status. The mean weight (kg) of school-aged children from household food secure and household food insecure were 39.98 ± 17.01 and 33.40 ± 16.24, respectively. The mean WAZ of school-aged children from household food secure and household food insecure were 0.75 ± 1.05 and −0.37 ± 1.20, respectively. The mean BMIZ of school-aged children from household food secure and household food insecure were 1.94 ± 0.57 ± 1.05 and −0.18 ± 1.09, respectively. Only 10.0 (9.3%) school-aged children from food-secure households experienced moderate underweight (weight for age), compared to 227 (83.5%) children from food-insecure households. Moreover, three children (2.7%) from food-secure households experienced severe underweight (weight for age), compared to 20.0 (7.3%) children from food-insecure households. Five children belonging to food-secure households experienced thinness, whereas 98 children belonging to food-insecure households experienced thinness. Besides, none of the children belonging to food-secure households experienced severe thinness, whereas ten children from food-insecure households experienced severe thinness. Furthermore, the results show significant association differences between household food security and household food insecurity concerning weight (kg), WAZ, BMIZ, underweight (weight for age), and malnutrition status ($P < 0.05$ for all).

On the other hand, **Table 3** shows energy, macro and micronutrients intakes of school-aged children by food security status. The results show that the mean levels of protein (gram) intake among school-aged children from household food secure and household food-insecure were 53.64 ± 22.83 and 40.54

TABLE 3 | Energy, macro and micronutrients intakes of school-aged children by food security status.

Variables	Household food-secure (n = 108)	Household food-insecure (n = 272)	P-value[a]
Energy (kcal)			
Mean ± SD	1,982 ± 791	1,948 ± 763	0.082
Protein (gram)			
Mean ± SD	53.64 ± 22.83	40.54 ± 24.57	**0.047***
Carbohydrate (gram)			
Mean ± SD	361.33 ± 170.14	385.63 ± 175.91	0.774
Fat (gram)			
Mean ± SD	76.73 ± 30.64	65.27 ± 32.54	0.068
Iron (mg)			
Mean ± SD	7.21 ± 2.96	6.12 ± 3.14	**0.036***
Vitamin A RAE (microgram)			
Mean ± SD	542.75 ± 245.04	512.51 ± 349.51	0.052
Vitamin D (microgram)			
Mean ± SD	11.24 ± 5.44	10.18 ± 5.57	**0.032***
Calcium (mg)			
Mean ± SD	1,045.02 ± 597.09	1,002.25 ± 599.41	0.063
Zinc (mg)			
Mean ± SD	5.79 ± 2.70	4.51 ± 2.38	**0.033***

[a]*Statistical testing using Independent samples t-test.*
**Difference is significant at the 0.05 level (two tailed).*
Difference is significant at the 0.05 level (two tailed).

± 24.57 g, respectively. The mean levels of iron (mg) intake among school-aged children from household food secure and household food-insecure were 7.21 ± 2.96 and 6.12 ± 3.14, respectively. The mean levels of vitamin D (μg) intakes among school-aged children from household food secure, and household food-insecure were 11.24 ± 5.44 and 10.18 ± 5.57, respectively. The mean levels of zinc intakes (mg) among school-aged children from household food secure, and household food-insecure were 5.79 ± 2.70 and 4.51 ± 2.38, respectively. The association differences found between household food security and household food insecurity regarding intakes of protein, iron, vitamin D, and zinc were significant ($P < 0.05$ for all).

Furthermore, **Figure 1** shows the difference between household food-secure and household food-insecure in relation to nutrition-related adequate knowledge. About 59.9 and 56.5% of food-secure households and food-insecure households had adequate knowledge about the importance of discouraging children intake of sweets and candies. The majority of food-secure households (80.9%) had adequate nutrition-related knowledge about the consequences of short-term hunger at school, whereas only 34.7% of food-insecure households had that knowledge.

Moreover, **Figure 2** shows the difference between household food-secure and household food-insecure concerning nutrition-related positive attitudes. The highest level of positive attitudes (83.6%) among household food security was related to having different types of foods at meal times-perceived benefit. The lowest level of positive attitudes (69.6%) was concerning having breakfast before going to school-perceived barriers. Moreover, the highest level of positive attitudes (56.2%) among household food insecure was related to having three meals a day and snacks-perceived benefits, while the lowest level of positive attitudes (36.1%) was concerning having breakfast before going to school-perceived benefits.

Additionally, **Figure 3** shows the difference between household food-secure and household food-insecure concerning nutrition-related good practices. The highest level of good practices (97.2%) among food-secure households was for the item related to (having lunch), and the lowest level of good practices (75.7%) concerned (time of breakfast). Furthermore, the highest level of good practices among the food insecure household (94.9%) was for the item related to (having lunch), and the lowest level of good practices (46.2%) was about bought food places.

Finally, **Table 4** shows the crude and adjusted odds ratio (OR) and 95% confidence interval (CI) for the overall nutrition related-KAP of school-age children by household food security status. The results revealed that 70.4, 76.9, and 84.3% of food secure households had nutrition-related adequate knowledge, positive attitudes, and good practices, respectively; while for the food-insecure households, the results for these criteria were 45.6, 46.7 and 70.6%, respectively. After adjustment for confounding variables, having nutrition-related adequate knowledge, positive attitudes, and good practices were associated with lower odds of being food insecure household, compared to those with inadequate knowledge, negative attitudes and bad practices [$OR = 0.519$, 95% CI = (0.320–0.841), $P < 0.05$], [$OR = 0.510$, 95% CI = (0.315–0.827), $P < 0.05$], and [$OR = 0.466$, 95% CI = (0.285–0.763), $P < 0.05$], respectively.

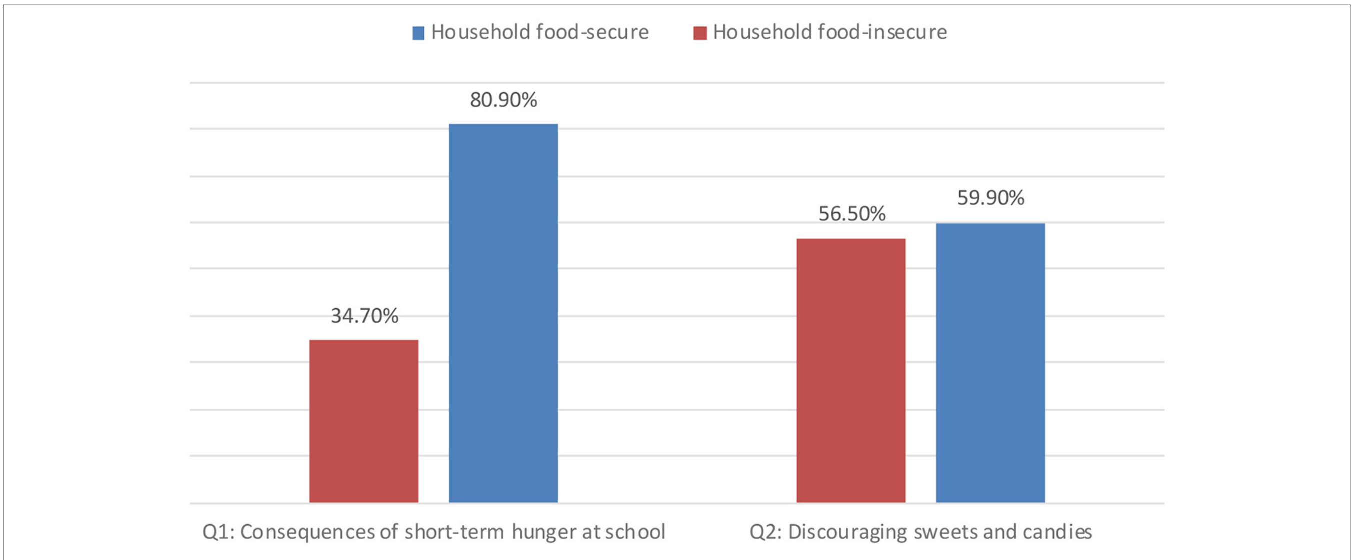

FIGURE 1 | Nutrition-related adequate knowledge among household food-secure and household food-insecure.

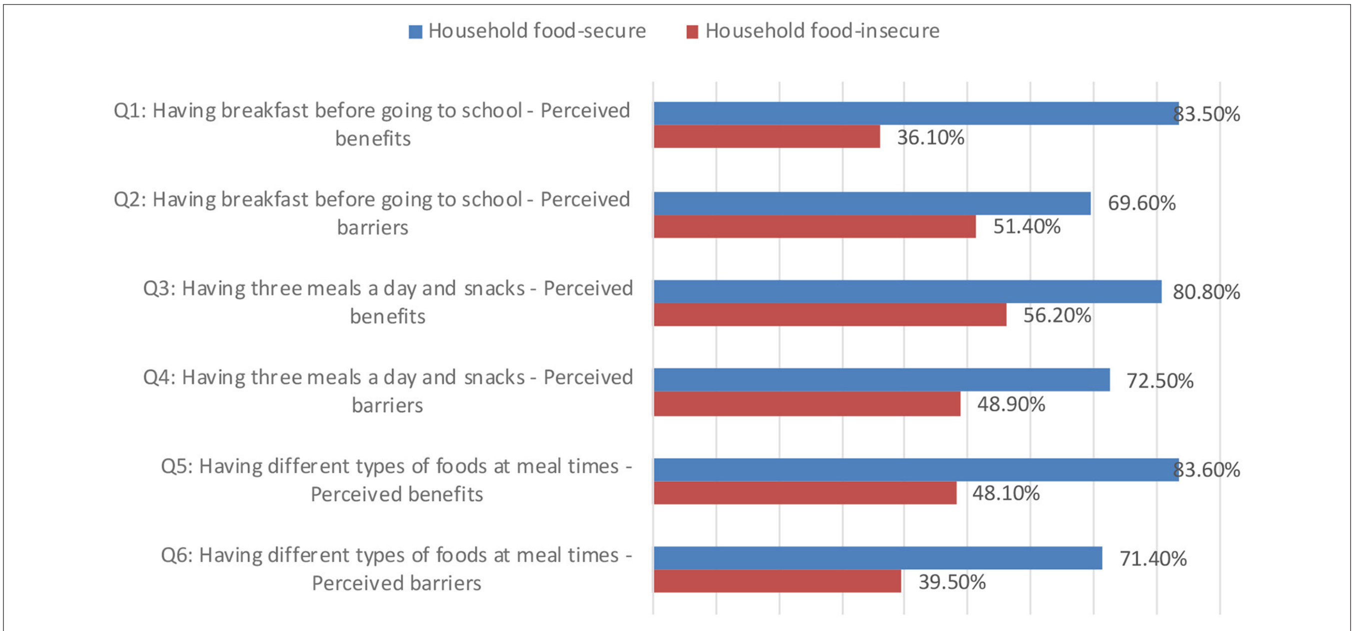

FIGURE 2 | Nutrition-related positive attitudes among household food-secure and household food-insecure.

DISCUSSION

To the best of our knowledge, this study was the first to evaluate the association of household food insecurity with dietary intakes and nutrition-related KAP among school-aged children in the Gaza Strip, Palestine. The findings of the present study may assist in establishing intervention programs aiming to improve the nutritional status of school-aged children experiencing food insecurity. More than two-thirds of our sample belonged to food-insecure households. Based on the recent data, over 68% of households in the Gaza strip are food-insecure (12). However, the results of our study are in line with the published data on some developing countries, such as Nepal (69%) (40) and Colombia (76%) (41). In addition, according to Elsahoryi et al. (42), 59.3% of Jordanian families were food insecure. The current analysis discovered a higher rate of food insecurity than the Food and Agriculture Organization (68.6%) (12). Food insecurity was prevalent in our study, which could be due to the Gaza Strip's extended war, economic stagnation, and limited trade and access to resources, as well as high unemployment and poverty rates (43).

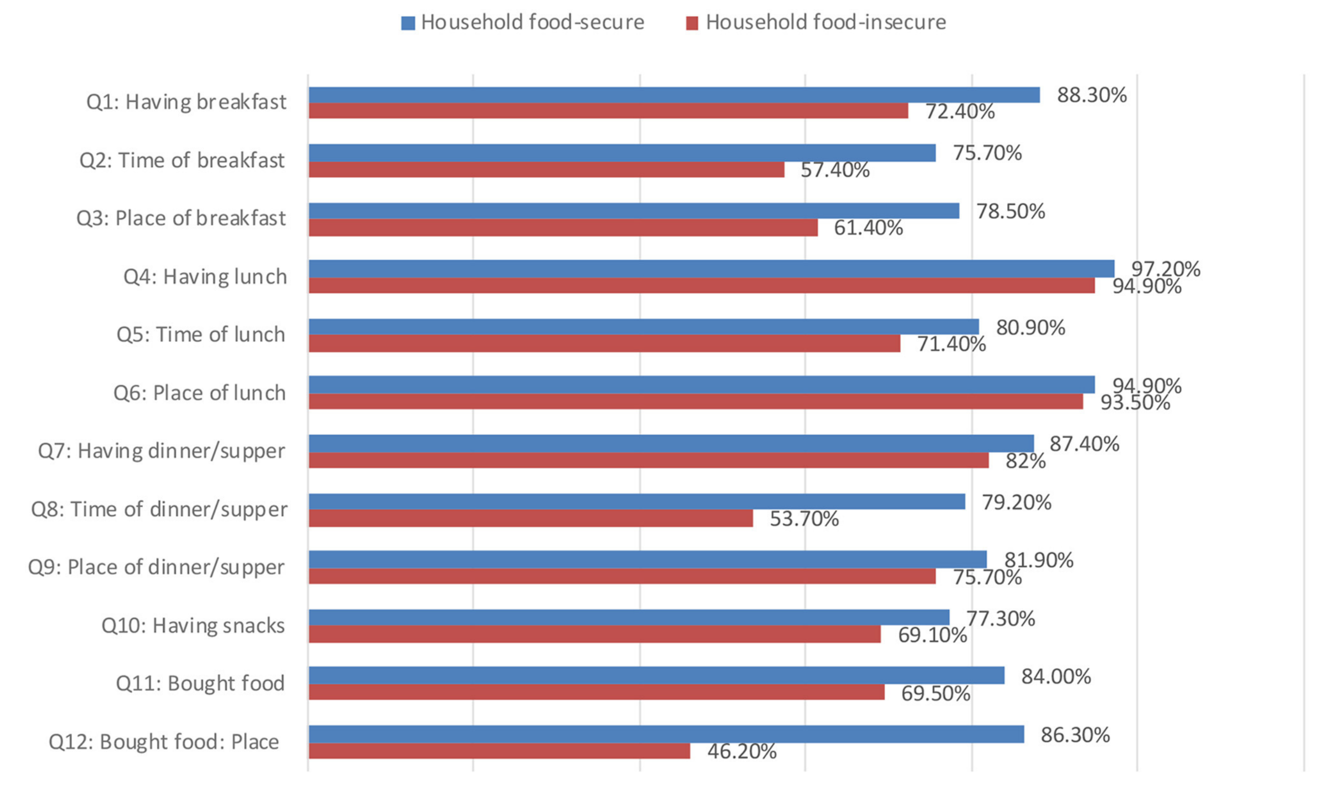

FIGURE 3 | Nutrition-related good practices among household food-secure and household food-insecure.

In the present study, households of school-aged children with food insecurity had significantly lower monthly incomes than that of secure food households. In addition, food-insecure households were significantly associated with being located in refugee camps and low parental education levels of children. Previous research has consistently found that the poor socioeconomic status of families is connected with food insecurity (44, 45). Recent research found that lower-income households had a higher frequency of food insecurity (43%) than higher-income households; the study also found that families with parents with a low educational level had a considerably higher incidence of food insecurity (46). Another study found that children from low-income families had greater levels of household food insecurity, had more crowded households, and had moms who were less educated (47). Economic status can affect household food security status and expenditure for food through its effect on food accessibility. The high levels of education of parents were shown to be strongly related to food security in Saudi Arabia; higher education offers prospects for better employment and wages; therefore, parents' education is important (48).

In this study, body weight (kg), WAZ, BMIZ, underweight (weight for age), and malnutrition status were found to be linked with food security status where the other measured anthropometric were not. In addition, the results demonstrated that school-aged children from food-insecure households have a low mean of weight (kg), a high risk of moderate and severely underweight, and a high risk of thinness and severe thinness. Earlier studies have also examined these associations, and outcomes were conflicting. For instance, a study conducted among children in the United States and Saudi Arabia investigated the association of food security with weight status, and no association was observed (48, 49). Similarly, a study conducted in Bogota, Colombia, reported that children from food-insecure households were three times as likely to be underweight, while stunting was not associated with food insecurity of the household (41). Other studies showed significant associations between food insecurity and childhood obesity (50) and stunted growth (51). These controversial findings can be explained by the different methods for food security assessment, age group differences, and the reference for nutritional status assessment.

In this study, school-aged children protein (g), iron (mg), vitamin D (μg), and zinc (mg) intakes were significantly lower in food-insecure households than in food-secure households. Household access to food depends on the purchasing power of their income. When the income level is low, households resort to a number of coping strategies such as reducing the size of the meal, reducing the number of meals eaten, and eating low quality or cheap foods to gain access to food and protect food security levels (52, 53). Thus, restrictions in food intake, if repeated over a long time, may explain the lower nutritional status indices

TABLE 4 | Crude and adjusted odds ratio and 95% confidence interval for the overall nutrition related-KAP of school-age children by household food-security status ($n = 380$).

Food security status	Household food secure		Statistical tests			
	Yes *n* (%) 108 (100)	No *n* (%) 272 (100)	Crude OR (95% CI)	*P*-value[a]	Adjusted OR (95% CI)[b]	*P*-value[a]
Have adequate knowledge						
No	32.0 (29.6)	148 (54.4)	Ref	–	–	**0.008***
Yes	76.0 (70.4)	124 (45.6)	0.803 (0.511–1.261)	0.340	0.519 (0.320–0.841)	
Have positive attitudes						
No	25.0 (23.1)	145 (53.3)	Ref	–	–	**0.006***
Yes	83.0 (76.9)	127 (46.7)	1.275 (0.799–2.034)	0.308	0.510 (0.315–0.827)	
Have good practices						
No	17.0 (15.7)	80 (29.4)	Ref	–	–	**0.002***
Yes	91.0 (84.3)	192 (70.6)	0.996 (0.794–1.249)	0.971	0.466 (0.285–0.763)	

[a] *Statistical testing using binary logistic regression.*
[b] *Adjusted for the living area, educational level of school-aged children, and monthly household income (NIS).*
[ref] *Reference.*
[*] *Difference is significant at the 0.05 level.*
Difference is significant at the 0.05 level (two tailed).

and lower nutrient intakes observed in the children living in food-insecure households.

On the other hand, a key finding in our study was that 70.4, 76.9, and 84.3% of food secure households had nutrition-related adequate knowledge, positive attitudes, and good practices, respectively; while for food-insecure households the values for these criteria were 45.6, 46.7 and 70.6%, respectively. Adequate nutrition-related KAP may be protective against food insecurity among school-aged children in the Gaza Strip, Palestine. Food insecurity is a significant nutritional issue worldwide and is commonly found in low- and middle- income countries like in Palestine. In addition, having physical and economic access to food on their own are not sufficient to ensure that people are food secure and well nourished. It is essential that people understand what constitutes a healthy diet; in particular, what nutrition-related health issues affect their communities, and how to address these through food-based approaches, and know how to make the best use of their resources. They should also have positive attitudes toward nutrition, diet, foods and closely related hygiene and health issues to be able to perform optimal dietary and feeding practices that ensure their nutritional wellbeing and that of their families. Finally, we found a positive association between parents' high level of education, and the high percentage of nutrition-related adequate knowledge, positive attitudes, and good practices with food security status of a household; may be this was possible because increased parents' education level could increase their knowledge about, attitude toward, and practice of healthy nutrition for the household members and specially for meeting the nutritional needs of the school-aged children.

Strengths and Limitations

The main strength of our study was no indication of selection bias in this study, which included a representative sample of school-aged children. A valid and reliable tool (Radimer/Cornell food security scale) was used for determining the household food security status. Two non-consecutive days of 24-h dietary recall were employed to determine the quantity of macro-and micronutrients consumed. A digital weighing scale (SECA, Germany) and stadiometer were used as measurement equipment for anthropometric indices. Furthermore, the survey included a standardized nutrition-related KAP questionnaire that was suggested by the FAO. The main limitation of this study is its cross-sectional design; the causal relationship could not be determined, and it limits the generalizability of our results. In addition, the possibility of recall bias and misreporting by using of self-report retrospective data of 24-h dietary recall for the assessment of food consumption are other limitations.

CONCLUSION

In conclusion, about 71.6% of school-aged children were in food-insecure households, and 28.4% were in food-secure households. Only 45.6, 46.7, and 70.6% of the food-insecure households had adequate nutrition-related knowledge, positive attitudes, and good practices, respectively. Additionally, low socioeconomic status, low anthropometric indices, poor dietary intakes may be associated with a high level of food insecurity, while having adequate nutrition-related KAP may be protective against food insecurity among school-aged children in the Gaza Strip, Palestine. Policy makers should continue to focus attention and investments in the most appropriate combinations of interventions to mitigate food insecurity level among school-aged children in the Gaza strip.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study protocol was approved by the Palestinian Health Research Council (Helsinki Ethical Committee of Research Number: PHRC/HC/961/21), University of Palestine Ethical Committee of Research, the Palestinian Ministry of Health, and Ministry of Interior. Further, written informed consent was obtained from each participant or their parents. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AE collected, analyzed, and interpreted the data and wrote the first draft of the manuscript. AE, AA-J, AA, SA, IE, and LN significantly contributed in the study design and the critical review of the manuscript. AE and AA-J remarkably contributed to the analysis and interpretation of data and the critical review of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.890850/full#supplementary-material

REFERENCES

1. FAO. *Hunger and food insecurity. Food and Agriculture Organization of the United Nations.* (2022). Available online at: https://www.fao.org/hunger/en/ (accessed March, 2022).
2. Coleman-Jensen AJ. US food insecurity status: toward a refined definition. *Soc Indic Res.* (2010) 95:215–30. doi: 10.1007/s11205-009-9455-4
3. World Health Organization. *The State of Food Security and Nutrition in the World 2020: Transforming Food Systems for Affordable Healthy Diets.* Geneva: Food & Agriculture Organization (2020).
4. UN. *High Costs, Persistent Poverty Prevent 3 Billion People from Accessing Healthy Diets, Secretary-General Says ahead of Food Systems Summit. United Nations*. (2021) SG/SM/20823. Available online at: https://www.un.org/press/en/2021/sgsm20823.doc.htm (accessed July 12, 2021)
5. FAO U, WFP, WHO. *In Brief to The State of Food Security and Nutrition in the World 2021. Transforming Food Systems for Food Security, Improved Nutrition and Affordable Healthy Diets for All*. Rome: Food And Agriculture Organization (2021).
6. Smith MD, Floro MS. Food insecurity, gender, and international migration in low-and middle-income countries. *Food Policy.* (2020) 91:101837. doi: 10.1016/j.foodpol.2020.101837
7. Jomaa L, Naja F, Kharroubi S, Hwalla N. Prevalence and correlates of food insecurity among Lebanese households with children aged 4–18 years: findings from a national cross-sectional study. *Public Health Nutr.* (2019) 22:202–11. doi: 10.1017/S1368980018003245
8. Wang Q, Awan MA, Ashraf J. The impact of political risk and institutions on food security. *Curr Res Nutr Food Sci.* (2020) 8:924. doi: 10.12944/CRNFSJ.8.3.21
9. OCHA. *Food insecurity in the oPt: 1.3 million Palestinians in the Gaza Strip Are Food Insecure.* The United Nations Office for the Coordination of Humanitarian Affairs. (2018) Available online at: https://www.ochaopt.org/content/food-insecurity-opt-13-million-palestinians-gaza-strip-are-food-insecure (accessed March, 2022).
10. AlKhaldi M, Abuzerr S, Obaid H, Alnajjar G, Alkhaldi A, Khayyat A. *Social Determinants of Health in Fragile and Conflict Settings: the Case of the Gaza Strip, Palestine. Handbook of Healthcare in the Arab World.* Cham: Springer (2020).p. 1–28. doi: 10.1007/978-3-319-74365-3_203-1
11. WFP. *Food Assistance for the Food-Insecure Population in the West Bank and Gaza Strip.* World Food Programme (2017) Available online at: https://www.wfp.org/operations/200709-food-assistance-food-insecure-population-west-bank-and-gaza-strip (accessed March, 2022).
12. FAO. *Socio-Economic and Food Security Survey 2018.* Food and Agriculture Organization (2018) Available online at: http://www.fao.org/3/cb0721en/CB0721EN.pdf (accessed March, 2022).
13. Faught EL, Williams PL, Willows ND, Asbridge M, Veugelers PJ. The association between food insecurity and academic achievement in Canadian school-aged children. *Public Health Nutr.* (2017) 20:2778–85. doi: 10.1017/S1368980017001562
14. Alaimo K, Olson CM, Frongillo EA. Food insufficiency and American school-aged children's cognitive, academic, and psychosocial development. *Pediatrics.* (2001) 108:44–53. doi: 10.1542/peds.108.1.44
15. Pilgrim A, Barker M, Jackson A, Ntani G, Crozier S, Inskip H, et al. Does living in a food insecure household impact on the diets and body composition of young children? Findings from the Southampton Women's Survey. *J Epidemiol Commun Health*. (2012) 66:e6–e. doi: 10.1136/jech.2010.125476
16. Blanchet R, Loewen OK, Godrich SL, Willows N, Veugelers P. Exploring the association between food insecurity and food skills among school-aged children. *Public Health Nutr.* (2020) 23:2000–5. doi: 10.1017/S1368980019004300
17. Marías Y, Glasauer P. *Guidelines for Assessing Nutrition-Related Knowledge, Attitudes and Practices:* Rome: Food and Agriculture Organization of the United Nations (FAO) (2014). p. 180
18. Weerasekara PC, Withanachchi CR, Ginigaddara G, Ploeger A. Food and nutrition-related knowledge, attitudes, and practices among reproductive-age women in marginalized areas in Sri Lanka. *Int J Environ Res Public Health.* (2020) 17:3985. doi: 10.3390/ijerph17113985
19. Mulu E, Mengistie B. Household food insecurity and its association with nutritional status of under five children in Sekela District, Western Ethiopia: a comparative cross-sectional study. *BMC Nutr.* (2017) 3:1–9. doi: 10.1186/s40795-017-0149-z
20. Vega-Macedo M, Shamah-Levy T, Peinador-Roldán R, Méndez-Gómez Humarán I, Melgar-Quiñónez H. Food insecurity and variety of food in Mexican households with children under five years. *Salud Publica Mex.* (2014) 56:s21–30. doi: 10.21149/spm.v56s1.5162
21. Eicher-Miller HA, Zhao Y. Evidence for the age-specific relationship of food insecurity and key dietary outcomes among US children and adolescents. *Nutr Res Rev.* (2018) 31:98–113. doi: 10.1017/S0954422417000245
22. Denney JT, Brewer M, Kimbro RT. Food insecurity in households with young children: a test of contextual congruence. *Soc Sci Med.* (2020) 263:113275. doi: 10.1016/j.socscimed.2020.113275
23. Melchior M, Caspi A, Howard LM, Ambler AP, Bolton H, Mountain N, et al. Mental health context of food insecurity: a representative cohort of families with young children. *Pediatrics.* (2009) 124:e564–e72. doi: 10.1542/peds.2009-0583
24. San Juan PF. Dietary habits and nutritional status of school aged children in Spain. *Nutr Hosp.* (2006) 21:374–8.
25. PCBS. *Statistic Brief (Palestinians at the end of 2016). Palestinian National Authority, Gaza, Palestine*. Palestinian Central Bureau of Statistics

(2016). Available online at: https://www.pcbs.gov.ps/post.aspx?langprotect%20\protect\T1\textdollarelax%20=\protect\T1\textdollaren&ItemIDprotect%20\protect\T1\textdollarelax%20=\protect\T1\textdollar1823 (accessed March, 2022).
26. Radimer KL, Olson CM, Greene JC, Campbell CC, Habicht J-P. Understanding hunger and developing indicators to assess it in women and children. *J Nutr Educ.* (1992) 24:36S—44. doi: 10.1016/S0022-3182(12)80137-3
27. FAO. *Guidelines for Assessing Nutrition-Related Knowledge, Attitudes and Practices*. Food and Agriculture Organization (2014). Available online at: http://www.fao.org/3/i3545e/i3545e00.htm (accessed July 24, 2021).
28. El Bilbeisi AH, Al-Jawaldeh A, Albelbeisi A, Abuzerr S, Elmadfa I, Nasreddine L. Households' food insecurity and its association with demographic and socioeconomic factors in Gaza Strip, Palestine: a cross-sectional study. *Ethiop J Health Sci.* (2022) 32:369–80. doi: 10.4314/ejhs.v32i2.18
29. Bilbeisi E, Hamid A, Al-Jawaldeh A, Albelbeisi A, Abuzerr S, Elmadfa I, et al. Households' food insecurity and their association with dietary intakes, nutrition-related knowledge, attitudes and practices among under-five children in Gaza Strip, Palestine. *Front Public Health*. (2022) 316:808700. doi: 10.3389/fpubh.2022.808700
30. Kendall A, Olson CM, Frongillo Jr EA. Relationship of hunger and food insecurity to food availability and consumption. *J Am Diet Assoc.* (1996) 96:1019–24. doi: 10.1016/S0002-8223(96)00271-4
31. Albelbeisi A, Shariff ZM, Mun CY, Rahman HA, Abed Y. Multiple micronutrient supplementation improves growth and reduces the risk of anemia among infants in Gaza Strip, Palestine: a prospective randomized community trial. *Nutr J.* (2020) 19:1–11. doi: 10.1186/s12937-020-00652-7
32. WHO. *The Use and Interpretation of Anthropometry-WHOP Status—WHO Technical Report Series*. Rome: World Health Organization (1995). p. 854.
33. El Bilbeisi AH, Hosseini S, Djafarian K. Association of dietary patterns with diabetes complications among type 2 diabetes patients in Gaza Strip, Palestine: a cross sectional study. *J Health Popul Nutr.* (2017) 36:37. doi: 10.1186/s41043-017-0115-z
34. World Health Organization. *WHO Anthro (Version 3.2. 2, January 2011) and Macros.* World Health Organization, Geneva, Switzerland (2011). Available online at: https://www.who.int/tools/child-growth-standards/software (accessed March, 2022).
35. WHO. Physical status: *The Use and Interpretation of Anthropometry: Report of a WHO Expert Committee.* Technical Report Series. Geneva: WHO (1995).p. 854.
36. WHO. *Growth Reference Data for 5–19 Years Indicators.* Available online at: https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age (accessed November, 2021).
37. Lohman TG, Roche AF, Martorell R. *Anthropometric Standardization Reference Manual*. Champaign, IL: Human Kinetics Books (1988).
38. FAO. *Food Safety Knowledge, Attitudes and Practices (KAP) Among Food Consumers in the West Bank and Gaza Strip*. Food and Agriculture Organization, Al Markaz for Development and Marketing Consultancies (2017). Available online at: https://www.fao.org/publications/card/ar/c/71236a64-5220-4ee9-9475-050b8abb886f/:75 (accessed March, 2022).
39. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian J Psychol Med.* (2013) 35:121. doi: 10.4103/0253-7176.116232
40. Osei A, Pandey P, Spiro D, Nielson J, Shrestha R, Talukder Z, et al. Household food insecurity and nutritional status of children aged 6 to 23 months in Kailali district of Nepal. *Food Nutr Bull.* (2010) 31:483–94. doi: 10.1177/156482651003100402
41. Isanaka S, Mora-Plazas M, Lopez-Arana S, Baylin A, Villamor E. Food insecurity is highly prevalent and predicts underweight but not overweight in adults and school children from Bogota, Colombia. *J Nutr.* (2007) 137:2747–55. doi: 10.1093/jn/137.12.2747
42. Elsahoryi N A-SH, Odeh M, McGrattan A, Hammad F. Effect of Covid-19 on food security: A cross-sectional survey. *Clin Nutr ESPEN.* (2020) 40:171–817. doi: 10.1016/j.clnesp.2020.09.026
43. Patterson K, Berrang-Ford L, Lwasa S, Namanya DB, Ford J, Twebaze F, et al. Seasonal variation of food security among the Batwa of Kanungu, Uganda. *Public Health Nutr.* (2017) 20:1–11. doi: 10.1017/S1368980016002494
44. Souza BFdNJd, Marin-Leon L, Camargo DFM, Segall-Correa AM. Demographic and socioeconomic conditions associated with food insecurity in households in Campinas, SP, Brazil. *Rev de Nutr*. (2016) 29:845–57. doi: 10.1590/1678-98652016000600009
45. Nagappa B, Rehman T, Marimuthu Y, Priyan S, Sarveswaran G, Kumar SG. Prevalence of food insecurity at household level and its associated factors in rural Puducherry: a cross-sectional study. *Indian J Community Med.* (2020) 45:303. doi: 10.4103/ijcm.IJCM_233_19
46. Orr CJ, Ben-Davies M, Ravanbakht SN, Yin HS, Sanders LM, Rothman RL, et al. Parental feeding beliefs and practices and household food insecurity in infancy. *Acad Pediatr.* (2019) 19:80–9. doi: 10.1016/j.acap.2018.09.007
47. Jamaluddine Z, Choufani J, Sahyoun NR, Ghattas H. A child-administered food security scale is associated with household socio-economic status, household food security and diet diversity. *FASEB J.* (2017) 31:297. doi: 10.1096/fasebj.31.1_supplement.297.2
48. Mumena WA, Kutbi HA. Household food security status, food purchasing, and nutritional health of Saudi girls aged 6-12 years. *Prog Nutr*. (2020) 22:1–12. doi: 10.23751/pn.v22i4.10424
49. Nguyen BT, Ford CN, Yaroch AL, Shuval K, Drope J. Food security and weight status in children: interactions with food assistance programs. *Am J Prev Med.* (2017) 52:S138–S44. doi: 10.1016/j.amepre.2016.09.009
50. Papas MA, Trabulsi JC, Dahl A, Dominick G. Food insecurity increases the odds of obesity among young Hispanic children. *J Immigr Minor Health.* (2016) 18:1046–52. doi: 10.1007/s10903-015-0275-0
51. Htet MK, Dibley M, Rammohan A, Vicol M, Pritchard B. Factors associated with stunting of under five-year children: findings from panel surveys in mountains, dry zone and delta regions of rural Myanmar (2016–17). *Curr Dev Nutr*. (2019) 3:39–19. doi: 10.1093/cdn/nzz051.P04-039-19
52. Brinkman H-J, De Pee S, Sanogo I, Subran L, Bloem MW. High food prices and the global financial crisis have reduced access to nutritious food and worsened nutritional status and health. *J Nutr*. (2010) 140:153–61S. doi: 10.3945/jn.109.110767
53. Norhasmah S, Zalilah M, Mohd Nasir M, Kandiah M, Asnarulkhadi A. A qualitative study on coping strategies among women from food insecurity households in Selangor and Negeri Sembilan. *Malays J Nutr*. (2010) 16:39–54.

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Dietary Zinc Intake Affects the Association Between Dietary Vitamin A and Depression: A Cross-Sectional Study

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Introduction: Dietary vitamin A concentrations correlate with depression. Zinc has been reported to be associated with lower depression. In addition, zinc is an important cofactor in the activation of vitamin A. However, there are few studies investigating relationships between of dietary zinc intake, dietary vitamin A intake and depression.

Materials and Methods: The data for this study came from the National Health and Nutrition Examination Survey (NHANES) from 2005 to 2018 and involved 70,190 participants. We stratified participants by recommended dietary zinc intake (recommended dietary zinc intake for women: 8 mg/day, recommended dietary zinc intake for men: 11 mg/day). We further assessed the association between vitamin A and depression in participants with low and high zinc intake (interaction test) using univariate logistic regression of intake participants.

Result: In the female population we grouped the population into low and high zinc intake groups using the recommended dietary zinc intake of 8 (mg/day), with an increase in total vitamin A, the risk of depression was significantly lower in the low zinc intake group (OR: 0.85 95 CI: 0.76–0.96), while the risk of depression was increased in the high zinc intake group (OR: 1.05 95 CI: 0.95 to 1.17). Thus, in the female population, there was a significant interaction between insufficient vitamin an intake and depression (interaction likelihood ratio test of $p = 0.011$). In the male population we grouped the population by the recommended dietary zinc intake of 11(mg/day). Again, the population was divided into two groups with low and high zinc intake, however we did not find significant results for the interaction ($p = 0.743$ for the interaction likelihood ratio test).

Conclusion: Our findings suggest that zinc intake may influence the relationship between dietary vitamin A and depression. Of course, our findings require further randomized controlled trials to enhance the credibility.

Keywords: cross-sectional study, vitamin A, depression, interaction, diet and nutrition

INTRODUCTION

In recent years, the increasing prevalence of depression has become a serious public health problem (1), which not only increases the associated morbidity and mortality, but in addition imposes a significant economic burden (2, 3). Therefore, it is essential to identify nutrients associated with depression to prevent its onset. Currently, vitamin A deficiency is becoming a common health problem (4). It is widely believed that vitamin A is an important raw material involved in the formation of light-sensitive substances within visual cells (5). In addition, vitamin A deficiency can cause dryness of the skin, conjunctiva and cornea, which can lead to severe dry eye disease and corneal ulcers, and this damage can involve epithelial tissues throughout the body, especially the respiratory, digestive and urinary tracts (6).

A possible link between vitamin A and depression has been identified in several recent research (7, 8). For example, a cohort study conducted by Bitarafan S showed a potential benefit of vitamin A intake in terms of reducing depression (7). In addition, a study conducted by Xue Y similarly showed that high intake of vitamin A significantly reduced the risk of depression (8). H In contrast, a study conducted by Hu P found that excessive vitamin A intake may increase the risk of depression and even suicidal tendencies (9). The discrepancy in the results of these studies may be due to inadequate consideration of potential confounding factors, like dietary zinc intake.

Zinc is an important regulator of the mammalian nervous and immune systems, a neurotransmitter of excitatory synapses in humans, and has an important role in stress responses and in the activation of zinc-dependent enzymes that maintain compensatory brain function (10). Previous studies have shown that zinc deficiency causes depression and anxiety-like behavior in humans, and that symptoms improve with zinc supplementation (11, 12). It has been suggested that zinc deficiency may also contribute to secondary vitamin A deficiency in the population, with a positive association between the two (13). However, clinical studies examining the effect of zinc intake on the association between vitamin A and depression are limited. In this cross-sectional study, we anticipated that zinc and vitamin A have an interaction effect on depression. The goal of this study was to investigate how zinc intake affected the link between vitamin A and depression.

MATERIALS AND METHODS

Data Sources and Study Population

Data from the National Health and Nutrition Examination Survey (NHANES) conducted consecutively from 2005 to 2018 were used in this study. In our study, participants aged 47 years or older who underwent an interview and examination at a Mobile Examination Center (MEC) were included. Participants without relevant covariates, depression were excluded, and those without a complete 24-h dietary recall were not included. In the National Health and Nutrition Examination Survey, noninstitutionalized citizens of the United States are assessed in terms of their health and dietary habits. In order to select a representative sample of survey participants, a multistage stratified probability design was used (14). At the MEC, the program conducted in-depth interviews to collect demographic and health history information, performed physical examinations, and collected blood samples. The samples were analyzed at the National Center for Environmental Health, Laboratory Sciences Division of the Centers for Disease Control and Prevention.

The National Ethical Review Board for Health Statistics Research approved the study. The original study protocol (protocol #2005-06; #2011-17), duly approved by the Ethics Review Board, is available on the NHANES Ethics Review Board website (https://www.cdc.gov/nchs/nhanes/irba98.htm). Our research is based on publicly available data from NHANES, all details are from the official website (https://www.cdc.gov/nchs/nhanes/about_nhanes.htm).

Measurement and Classification of Dietary Vitamin A and Zinc Dietary Intake

A dietary recall interview at the Mobile Examination Center (MEC)was used to collect information on zinc intake and vitamin A intake in the past 24 h. The dietary interview component is called What We Eat in America (WWEIA), and What We Eat in America data were collected using USDA's dietary data collection instrument, the Automated Multiple Pass Method (AMPM), available at: http://www.ars.usda.gov/nea/bhnrc/fsrg. The AMPM, providing accurate estimates of population intake (15, 16), was designed to provide an efficient and accurate means of collecting intakes for large-scale national surveys. The AMPM is a fully computerized recall method that uses a 5-step interview outlined below:

1. Quick List-Participant recalls all foods and beverages consumed the day before the interview (midnight to midnight).
2. Forgotten Foods - Participant is asked about consumption of foods commonly forgotten during the Quick List step.
3. Time and Occasion - Time and eating occasion are collected for each food.
4. Detail Cycle - For each food, a detailed description, amount eaten, and additions to the food are collected. Eating occasions and times between eating occasions are reviewed to elicit forgotten foods.
5. Final Probe - Additional foods not remembered earlier are collected.

All NHANES participants are eligible for two 24-h dietary recall interviews. The first dietary recall interview is collected in-person in the Mobile Examination Center (MEC) and the second interview is collected by telephone 3 to 10 days later. However, in order to ensure the accuracy of the data, the first dietary recall interview was chosen by our reach.

We stratified participants by recommended dietary zinc intake (recommended dietary zinc intake for women: 8 mg/day, recommended dietary zinc intake for men: 11 mg/day). Dietary vitamin A is a continuous variable, and its subgroups are grouped according to the median value. The decision to continue using this method in NHANES was based on consensus reached by a panel of experts at a regular workshop to evaluate NHANES data collection methods (17).

Zinc intake and vitamin A intake acquisition and measurement can be found in the NHANES database (https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/DR1IFF_D.htm).

Depression Classification

Depression was defined based on PHQ-9 criteria and self-report questionnaires (18, 19).The Patient Health Questionnaire-9 (PHQ-9) is a self-rating scale for depressive disorders that is based on the nine entries of the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders developed by the American Psychiatric Association) diagnostic criteria, and has high reliability and validity for both diagnosing depression and determining symptom severity. Depressed participants were defined as those who satisfied the following criteria: depression scores in the 0–4 range for not having depression and in the 5–15 range for having depression (18).

Covariate

Age, gender, race/ethnicity, marriage, household income, body mass index (BMI), education level, smoking status, physical activity, work activity, alcohol consumption status, diabetes, and hypertension were considered as potential confounders in this study. Participants were self-classified on their race/ethnicity. Poverty Income Ratio (PIR) means a radio of family income to poverty, which can be found on the official website of NHANES (https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/DEMO_H.htm#INDFMPIR). Marital status was categorized as married or unmarried, where separated, divorced, cohabiting, married, and widowed were defined as married. Not having completed high school, having completed high school, and having completed college and above were the three levels of education. Current smokers, former smokers, and non-smokers were the three smoking categories. Current smokers were those who had smoked more than 100 cigarettes in the past and reported smoking for a few days or daily at the time of the interview. Ex-smokers were defined as people who had smoked more than 100 cigarettes in their lives but were no longer smokers. Nonsmokers were defined as those who had never smoked more than 100 cigarettes in their lives. According to the standardized protocol, BMI was calculated based on weight and height. Physical activity was classified into three levels according to the intensity of activity: non-work activity, moderate work activity and vigorous work activity. BMI was calculated based on measured height and weight. BMI was computed using height and weight measurements. On a digital scale, weight was measured in pounds and then converted to kilograms. Height was measured to the nearest millimeter using an electronic rangefinder.

If one of the following criteria is met, it can be judged as hypertension or diabetes. The definition criteria of diabetes are as follows: (1) doctor told you to have diabetes (2) Self-reported diabetes for a long time (3) glycohemoglobin HbA1c (%) >6.5 (4) the fasting glucose (mmol/L) ≥7.0 (5) random blood glucose (mmol/L) ≥11.1 (6) 2-h OGTT blood glucose (mmol/L) ≥11.1 (7) Use of diabetes medication or insulin (8) diabetes at birth is considered type 1 diabetes. Hypertension case definitions are based on the International Society of High Blood Pressure standards and a self-reported questionnaire. Participants were identified as hypertensive if they met the following criteria: (1) current use of hypertension medication (2) based on accurate diagnosis by the physician (3) based on blood pressure measured in real-time ≥140/90 mmHg (4) self-reported questionnaire data showing physician's prior diagnosis of hypertension and current use of medication to lower blood pressure. (5) The diagnostic criteria for hypertension by ambulatory blood pressure monitoring (ABPM) were: mean blood pressure ≥130/80 mmHg within 24 h, daytime ≥135/85 mmHg, at night ≥120/70 mmhg. Alcohol Data on alcohol drinking (yes = a minimum of 12 alcoholic beverages every year vs. no = fewer than 12 alcoholic beverages per year) was obtained by questionnaire interviews.

Statistical Analysis

The statistical software R (http://www.R-project.org, The R Foundation) was used to conduct all of the analyses. For the stratified sampling data extracted from the nhanes database we used the statistical methods of multiple logistic regression analysis, stratified analysis, and sensitivity analysis (https://wwwn.cdc.gov/nchs/nhanes/AnalyticGuidelines.aspx). A multivariate linear regression approach was used to investigate the link between vitamin A and depression. Depression values were assessed at various levels of zinc intake. Interactions between subgroups were examined by likelihood ratio tests. Ninety-five percentage confidence intervals (Cls) were calculated. The statistical significance level was set at 0.05. Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) in descriptive analysis, and categorical variables as weighted percentages (%). To examine continuous and categorical variables, the chi-square test (categorical variables), *t*-test (normal distribution), and Kruskal-Wallis (skewed distribution) tests were used.

RESULT

Baseline Characteristics of the Study Population

Seven cycles of NHANES 2005–2006, 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018 were used in this study. There were 70,190 participants in this study, of whom 31,839 adults (≥18 years old) completed the interview, and our study also included the MEC examination. Participants with unknown classification regarding depression ($n = 33,853$) were excluded. After excluding participants with missing covariate data, a total of 31,839 participants were included in our analysis (**Figure 1**). A summary of the overall plot of exclusion criteria is shown in **Figure 1**. The descriptive characteristics of the participants according to their depression are shown in **Table 1**. Compared to the non-depression, participants with depression were more likely to be elder, male, non- Hispanic white, in the state of marriage, had higher BMI, had a higher level of education, PIR>1, lower intake of smoke, less physical activity, less work

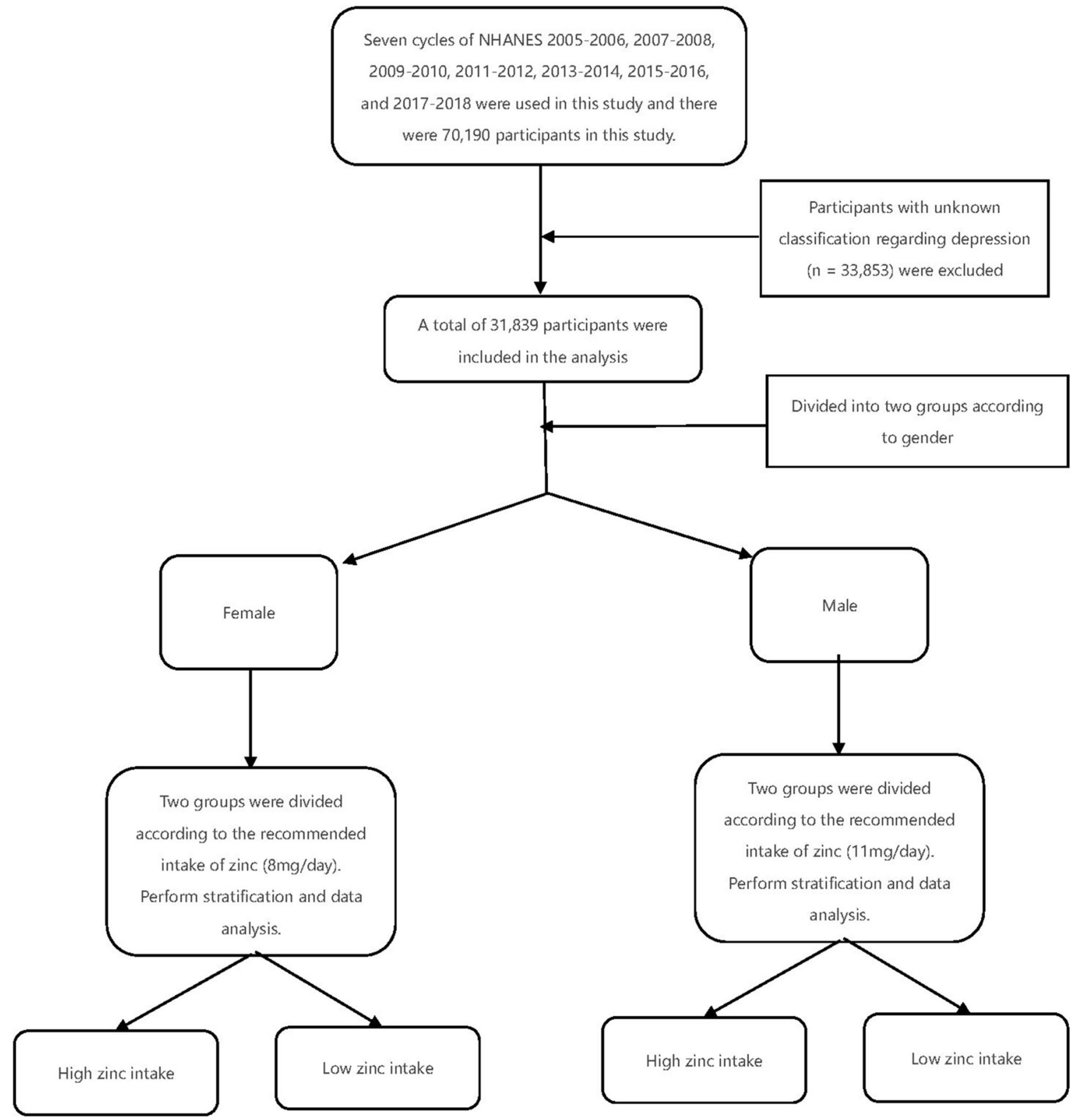

FIGURE 1 | Experimental methods and procedures.

activity. There were no statistically significant differences in drinking status ($P > 0.05$).

Association of Dietary Vitamin A With Depression

Among women, vitamin A was negatively associated with depression in the low-zinc group. However, in the high-zinc group, vitamin A was not associated with depression after adjusting for confounders ($p > 0.05$). This phenomenon was only observed in females.

Zinc Intake Affects the Association Between Vitamin A and Depression

In the female population we grouped the population into low and high zinc intake groups using the recommended dietary zinc intake 8 (mg/day). The risk of depression was significantly

TABLE 1 | Baseline characteristics of study participants.

Variables	Depressive state			*p*-value
	Total (*n* = 31,839)	**Depression (*n* = 15,901)**	**Non-depression (*n* = 15,938)**	
Age, Median (IQR)	47.0 (31.0, 63.0)	47.0 (31.0, 63.0)	47.0 (31.0, 61.0)	0.004
Gender, *n* (%)				< 0.001
Female	16,145 (50.7)	11,460 (47.7)	4,685 (60.1)	
Male	15,694 (49.3)	12,584 (52.3)	3,110 (39.9)	
Race, n (%)				< 0.001
Mexican American	4,986 (15.7)	3,775 (15.7)	1,211 (15.5)	
Non-Hispanic black	6,851 (21.5)	5,166 (21.5)	1,685 (21.6)	
Non-Hispanic white	13,953 (43.8)	10,518 (43.7)	3,435 (44.1)	
Other Hispanic	2,845 (8.9)	2,038 (8.5)	807 (10.4)	
Other race-including multiracial	3,204 (10.1)	2,547 (10.6)	657 (8.4)	
Marital status, *n* (%)				< 0.001
No	5,783 (18.2)	4,194 (17.4)	1,589 (20.4)	
Yes	24,693 (77.6)	18,841 (78.4)	5,852 (75.1)	
Unknown	1,363 (4.3)	1,009 (4.2)	354 (4.5)	
PIR, Mean ± SD	2.5 ± 1.6	2.7 ± 1.6	2.0 ± 1.5	< 0.001
BMI, *n* (%)				< 0.001
<25	9,494 (29.8)	7,412 (30.8)	2,082 (26.7)	
25–29.9	10,420 (32.7)	8,224 (34.2)	2,196 (28.2)	
>30	11,925 (37.5)	8,408 (20)	3,517 (45.1)	
Educational level, *n* (%)				< 0.001
Less than high school	7,563 (23.8)	5,236 (21.8)	2,327 (29.9)	
High school graduation	7,601 (23.9)	5,615 (23.4)	1,986 (25.5)	
College or above	16,675 (52.4)	13,193 (54.9)	3,482 (44.7)	
Smoking status, *n* (%)				< 0.001
Never	16,941 (53.2)	13,437 (55.9)	3,504 (45)	
Former	7,448 (23.4)	5,701 (23.7)	1,747 (22.4)	
Now	6,280 (19.7)	4,021 (16.7)	2,259 (21)	
Unknown	1,170 (3.7)	885 (3.7)	285 (3.7)	
Physical status, *n* (%)				< 0.001
NO/Unknown	18,363 (57.7)	13,200 (54.9)	5,163 (66.2)	
Moderate	7,012 (22.0)	5,516 (22.9)	1,496 (19.2)	
Vigorous	6,464 (20.3)	5,328 (22.2)	1,136 (14.6)	
Work activity, *n* (%)				< 0.001
Non-work activity	15,727 (49.4)	11,776 (49)	3,951 (50.7)	
Moderate work activity	6,076 (19.1)	4,545 (18.9)	1,531 (19.6)	
Vigorous work activity	5,563 (17.5)	4,159 (17.3)	1,404 (18)	
Unknown	4,473 (14.0)	3,564 (14.8)	909 (11.7)	
Alcohol, *n* (%)				0.119
No	7,635 (24.0)	5,814 (24.2)	1,821 (23.4)	
Yes	19,069 (59.9)	14,402 (59.9)	4,667 (59.9)	
Unknown	5,135 (16.1)	3,828 (15.9)	1,307 (16.8)	
DM, *n* (%)				< 0.001
No	23,779 (74.7)	18,250 (75.9)	5,529 (70.9)	
Yes	8,060 (25.3)	5,794 (24.1)	2,266 (29.1)	
Hypertension, *n* (%)				< 0.001
No	19,342 (60.7)	15,064 (62.7)	4,278 (54.9)	
Yes	12,497 (39.3)	8,980 (37.3)	3,517 (45.1)	
Vit A intake, Median (IQR)	465.0 (252.0, 770.0)	479.0 (262.0, 782.0)	427.0 (224.5, 731.5)	< 0.001
Zn intake, Median (IQR)	9.7 (6.6, 14.1)	9.9 (6.8, 14.2)	9.2 (6.1, 13.7)	< 0.001

reduced with increasing total vitamin A intake in the low zinc intake group (OR: 0.85 95 CI%: 0.76–0.96), while the risk of depression in the high zinc intake group (OR: 1.05 95 CI%:0.95–1.17), thus, there was a significant interaction between insufficient vitamin A intake and depression in the female population (The interaction likelihood ratio test was $p = 0.011$). In the male population, we grouped the population with the recommended dietary zinc intake of 11(mg/day), and similarly, divided the population into two groups with low and high zinc intake, however, we did not find a significant interaction (p=0.743 for the interaction likelihood ratio test). According to **Table 2**, the *p*-value of β remained stable, while in the high Zinc intake group, most of the *P*-values of β are not statistically significant in the low Zinc intake group.

DISCUSSION

In our study, we found that vitamin A and low dietary zinc intake were significantly associated with a reduced prevalence of depression in a female population. This association remained significant after adjusting for confounding. In NHANES, the study sample size is quite large, and the quality is authoritative and strictly quality controlled. One-day of dietary intake data has been shown to be adequate for estimating and comparing mean intakes of population groups. (https://dietassessmentprimer.cancer.gov/profiles/recall) So our findings can be applied to all populations.

According to reports, depressed patients are commonly deprived of zinc (22, 23). And it has been reported that zinc intake is associated with a low prevalence of depression (23, 24). We divided the population by recommended dietary zinc intake into low and high zinc intake, and separated the results by gender. Our results suggest that zinc supplementation is effective in a range of depressed patients in the female population. However, it is worth noting that excessive zinc intake does not further reduce the risk of depression and does not hold for the male population, which is consistent with other reports (25, 26).

To our knowledge, only a few studies have looked at the effect of dietary zinc on the relationship between dietary vitamin A and depression. Similar to our findings, Qian Yao used the 24-item Hamilton Depression Scale to see if serum retinol-binding protein 4 (RBP4) concentrations may change depression symptoms in individuals (27). In the blood, RBP4 acts as a specific transporter protein for the micronutrient, vitamin A This research highlights the potential importance of adequate nutritional vitamin A status for adult brain function, owing to their role in the regulation of synaptic plasticity, as well as associated learning and memory behaviors, which may be a major factor in mood disorders such as major depression. The same conclusion was given by Farhadnejad H in his research (28). RBP4 can be transferred from the circulation system to the cerebro-spinal fluid and bind to retinol, thus facilitating the metabolism of retinol to retinoic acid in neuroepithelial cells (29). In turn, retinoic acid plays many important roles in regulating neurons, such as in plasticity, regeneration, differentiation, learning and memory (30).

According to our results, the relationship between zinc intake and depression is valid only in the female population and does not hold for men, which is in line with other reports (25, 26). Of course, the mechanisms involved have not yet been clearly elucidated. But in one study we learned of a possibility (26). First, according to current research, gender is an important factor affecting the clinical presentation and prognosis of depression (21, 31, 32). And according to the general research (33–35), the prevalence of depression is significantly higher in female than in male. This study considered that sex differences in

TABLE 2 | Effect of low and high zinc intake groups on the association between vitamin A and depression in dichotomous and trichotomous models.

Female (model 6)					
Variable	**Dietary intake zinc≤8(mg/d) (*n* = 7,541)**		**Dietary intake zinc>8(mg/d) (*n* = 8,604)**		***P* for interaction**
Vitamin A	**OR(95 CI%)**	***P*-value**	**OR(95 CI%)**	***P*-value**	
Subgroups					0.011
≤440 (mcg)	1(reference)		1(reference)		
>440 (mcg)	0.85 (0.76∼0.96)	0.007	1.05 (0.95∼1.17)	0.331	
Male (model 6)					
Variable	**Dietary intake zinc≤11(mg/d) (*n* = 7,397)**		**Dietary intake zinc>11(mg/d) (*n* = 8,297)**		**P for interaction**
Vitamin A	**OR(95 CI%)**	***P*-value**	**OR(95 CI%)**	***P*-value**	
Subgroups					0.743
≤495 (mcg)	1(reference)		1(reference)		
>495 (mcg)	0.97 (0.85∼1.11)	0.679	1 (0.89∼1.13)	0.952	

Covariables include age, race, education level, PIR, marriage status, BMI, physical activity, work activity, smoking status, alcohol, DM and Hypertension.
Model 6: adjust for age, race, education level, PIR, marriage status, BMI, physical activity, work activity, smoking status, alcohol, DM, Hypertension.

neural structural and neurological functional parameters may be a factor associated with depressive symptoms of which gender differences in some serotonergic systems might play a role in the pathophysiology of depression (20). And it has been suggested that certain processes of the serotonin system may be more pronounced in women than in men (36, 37). This phenomenon may explain the interaction of zinc across genders in the relationship between vitamin A and depression.

However, a cross-sectional study showed that vitamin A can be an effective prevention (38), but not a treatment, for depression. It may be due to the study population with lower zinc intake. A study has shown that vitamin A and zinc deficiency usually occur at the same time because zinc deficiency reduces plasma retinol concentrations and reduces the production of retinol-binding proteins (39). Thus, zinc intake facilitates the potentiation of vitamin A activity. This impact could account for the interaction effect observed in our research. Given the nature of depression's etiological process, as well as the fact that depression is accompanied by an inflammatory response, including an increase in pro-inflammatory cytokines and lipid peroxidation (40), recent evidence supports an association between lipid peroxidation and major depression (41), and dietary antioxidants have the potential to play an important role in the prevention and treatment of depression. However, one study have shown that excessive intake of vitamin A can cause acute or chronic toxic effects (42). Of course, toxicity is only statistically possible after consuming 20 times more than the recommended intake of the vitamin and for several months. But perhaps it is the zinc intake that causes the increased activity of vitamin A, which lowers the threshold for vitamin A toxicity. Therefore, through our study, we believe that it is necessary to control the amount of zinc when using large amounts of vitamin A in the treatment of depression. This of course needs to be confirmed in further clinical trials.

In addition, one study found that venlafaxine treatment may reduce retinol binding protein 4 (RBP-4) levels. And the level of RBP4 in patients with major depressive depression (MDD) was lower than normal people (27). Therefore, it is necessary to take into account the effects of venlafaxine when considering the treatment of depression.

Our study still has some limitations. First, because of the cross-sectional design, we were unable to prove causality or directionality. Even after multiple adjustment, the results may be confounded by some other variables that were not measured. Nevertheless, some potential confounders were adjusted for in the logistic regression model, including some dietary factors. Second, there is no easy and exact way to measure total body zinc status. We obtained the zinc intake of participants by a dietary interview/24-h recall. Since dietary data were obtained from self-reported 24-h dietary recall, recall bias is difficult to avoid. Third, although a large sample was included in this study, the study population included only US residents. Therefore, practical considerations need to be taken into account when extrapolating to other populations. Therefore, well-designed multicenter controlled trials are needed to validate our findings.

CONCLUSION

Conclusively, results of this study suggest that zinc intake may have an impact on the relationship between dietary vitamin A and depression. Despite providing clinical insight by this trial, more randomized controlled studies are required to provide more data.

DATA AVAILABILITY STATEMENT

NHANES has developed a public use dataset, available at: https://www.cdc.gov/nchs/nhanes/index.htm. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

AUTHOR CONTRIBUTIONS

BH and Z-yL: conception and design and provision of study materials or patients. MY and D-lS: administrative support. Y-wG and R-pZ: collection and assembly of data. All authors: data analysis and interpretation, manuscript writing, and final approval of manuscript.

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REFERENCES

1. Ferrari AJ, Somerville AJ, Baxter AJ, Norman R, Patten SB, Vos T, et al. Global variation in the prevalence and incidence of major depressive disorder: a systematic review of the epidemiological literature. *Psychol Med.* (2013) 43:471–81. doi: 10.1017/S0033291712001511
2. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the world health surveys. *Lancet.* (2007) 370:851–8. doi: 10.1016/S0140-6736(07)61415-9
3. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. *Lancet.* (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9
4. Sherwin JC, Reacher MH, Dean WH, Ngondi J. Epidemiology of vitamin a deficiency and xerophthalmia in at-risk populations. *Trans R Soc Trop Med Hyg.* (2012) 106:205–14. doi: 10.1016/j.trstmh.2012.01.004
5. Dawson MI. The importance of vitamin a in nutrition. *Curr Pharm Des.* (2000) 6:311–25. doi: 10.2174/1381612003401190
6. Roche FC, Harris-Tryon TA. Illuminating the role of vitamin a in skin innate immunity and the skin microbiome: a narrative review. *Nutrients.* (2021) 13:302. doi: 10.3390/nu13020302
7. Bitarafan S, Saboor-Yaraghi A, Sahraian MA, Soltani D, Nafissi S, Togha M, et al. Effect of vitamin a supplementation on fatigue and depression in multiple sclerosis patients: a double-blind placebo-controlled clinical trial. *Iran J Allergy Asthma Immunol.* (2016) 15:13–9.
8. Xue Y, Zeng M, Zheng YI, Wang L, Cao S, Wu C, et al. Gender difference in vitamin a levels in first-episode drug-naïve depression patients:

a case-control and 24-weeks follow-up study. *Pharmazie.* (2020) 75:32–5. doi: 10.1691/ph.2020.9829

9. Hu P, van Dam AM, Wang Y, Lucassen PJ, Zhou JN. Retinoic acid and depressive disorders: evidence and possible neurobiological mechanisms. *Neurosci Biobehav Rev.* (2020) 112:376–91. doi: 10.1016/j.neubiorev.2020.02.013
10. Petrilli MA, Kranz TM, Kleinhaus K, Joe P, Getz M, Johnson P, et al. The emerging role for zinc in depression and psychosis. *Front Pharmacol.* (2017) 8:414. doi: 10.3389/fphar.2017.00414
11. Wang J, Um P, Dickerman BA, Liu J. Zinc, magnesium, selenium and depression: a review of the evidence, potential mechanisms and implications. *Nutrients.* (2018) 10:584. doi: 10.3390/nu10050584
12. Siodłak D, Nowak G, Mlyniec K. Interaction between zinc, the Gpr39 zinc receptor and the serotonergic system in depression. *Brain Res Bull.* (2021) 170:146–54. doi: 10.1016/j.brainresbull.2021.02.003
13. Sweetman DU, O'Donnell SM, Lalor A, Grant T, Greaney H. Zinc and vitamin a deficiency in a cohort of children with autism spectrum disorder. *Child Care Health Dev.* (2019) 45:380–6. doi: 10.1111/cch.12655
14. Zipf G, Chiappa M, Porter KS, Ostchega Y, Lewis BG, Dostal J. *National Health and Nutrition Examination Survey: Plan and Operations, 1999-2010.* Vital and health statistics Ser 1, Programs and collection procedures (2013). p. 1–37.
15. Rhodes DG, Murayi T, Clemens JC, Baer DJ, Sebastian RS, Moshfegh AJ. The USDA automated multiple-pass method accurately assesses population sodium intakes. *Am J Clin Nutr.* (2013) 97:958–64. doi: 10.3945/ajcn.112.044982
16. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The US department of agriculture automated multiple-pass method reduces bias in the collection of energy intakes. *Am J Clin Nutr.* (2008) 88:324–32. doi: 10.1093/ajcn/88.2.324
17. Ahluwalia N, Dwyer J, Terry A, Moshfegh A, Johnson C. Update on nhanes dietary data: focus on collection, release, analytical considerations, and uses to inform public policy. *Advances in Nutrition.* (2016) 7:121–34. doi: 10.3945/an.115.009258
18. Levis B, Benedetti A, Thombs BD. Accuracy of Patient Health Questionnaire-9 (Phq-9) for Screening to Detect Major Depression: Individual Participant Data Meta-Analysis. *BMJ (Clinical research ed).* (2019) 365:l1476. doi: 10.1136/bmj.l1476
19. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of prime-Md: the Phq primary care study. Primary care evaluation of mental disorders patient health questionnaire. *JAMA.* (1999) 282:1737–44. doi: 10.1001/jama.282.18.1737
20. Halbreich U, Lumley LA. The multiple interactional biological processes that might lead to depression and gender differences in its appearance. *J Affect Disord.* (1993) 29:159–73. doi: 10.1016/0165-0327(93)90030-N
21. Parker G, Fletcher K, Paterson A, Anderson J, Hong M. Gender differences in depression severity and symptoms across depressive sub-types. *J Affect Disord.* (2014) 167:351–7. doi: 10.1016/j.jad.2014.06.018
22. Maes M, D'Haese PC, Scharpé S, D'Hondt P, Cosyns P, De Broe ME. Hypozincemia in depression. *J Affect Disord.* (1994) 31:135–40. doi: 10.1016/0165-0327(94)90117-1
23. Swardfager W, Herrmann N, Mazereeuw G, Goldberger K, Harimoto T, Lanctôt KL. Zinc in depression: a meta-analysis. *Biol Psychiatry.* (2013) 74:872–8. doi: 10.1016/j.biopsych.2013.05.008
24. Vashum KP, McEvoy M, Milton AH, McElduff P, Hure A, Byles J, et al. Dietary zinc is associated with a lower incidence of depression: findings from two australian cohorts. *J Affect Disord.* (2014) 166:249–57. doi: 10.1016/j.jad.2014.05.016
25. Maserejian NN, Hall SA, McKinlay JB. Low dietary or supplemental zinc is associated with depression symptoms among women, but not men, in a population-based epidemiological survey. *J Affect Disord.* (2012) 136:781–8. doi: 10.1016/j.jad.2011.09.039
26. Thi Thu Nguyen T, Miyagi S, Tsujiguchi H, Kambayashi Y, Hara A, Nakamura H, et al. Association between lower intake of minerals and depressive symptoms among elderly japanese women but not men: findings from shika study. *Nutrients.* (2019) 11:389. doi: 10.3390/nu11020389
27. Yao Q, Li Y. Study of decreased serum levels of retinol binding protein 4 in major depressive disorder. *J Psychiatr Res.* (2020) 129:24–30. doi: 10.1016/j.jpsychires.2020.05.030
28. Farhadnejad H, Neshatbini Tehrani A, Salehpour A, Hekmatdoost A. Antioxidant vitamin intakes and risk of depression, anxiety and stress among female adolescents. *Clin Nutr ESPEN.* (2020) 40:257–62. doi: 10.1016/j.clnesp.2020.09.010
29. Chang JT, Lehtinen MK, Sive H. Zebrafish cerebrospinal fluid mediates cell survival through a retinoid signaling pathway. *Dev Neurobiol.* (2016) 76:75–92. doi: 10.1002/dneu.22300
30. Maden M. Retinoic acid in the development, regeneration and maintenance of the nervous system. *Nature Reviews Neuroscience.* (2007) 8:755–65. doi: 10.1038/nrn2212
31. Shim RS, Baltrus P, Ye J, Rust G. Prevalence, treatment, and control of depressive symptoms in the United States: results from the national health and nutrition examination survey (Nhanes), 2005-2008. *JABFM.* (2011) 24:33–8. doi: 10.3122/jabfm.2011.01.100121
32. Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, et al. Gender differences in treatment response to sertraline versus imipramine in chronic depression. *Am J Psychiatry.* (2000) 157:1445–52. doi: 10.1176/appi.ajp.157.9.1445
33. Blazer DG, Kessler RC, McGonagle KA, Swartz MS. The prevalence and distribution of major depression in a national community sample: the national comorbidity survey. *Am J Psychiatry.* (1994) 151:979–86. doi: 10.1176/ajp.151.7.979
34. Van de Velde S, Bracke P, Levecque K. Gender differences in depression in 23 European Countries. Cross-national variation in the gender gap in depression. *Soc Sci Med.* (2010) 71:305–13. doi: 10.1016/j.socscimed.2010.03.035
35. Schuch JJ, Roest AM, Nolen WA, Penninx BW, de Jonge P. Gender differences in major depressive disorder: results from the Netherlands study of depression and anxiety. *J Affect Disord.* (2014) 156:156–63. doi: 10.1016/j.jad.2013.12.011
36. Chang CC, Chang HA, Fang WH, Chang TC, Huang SY. Gender-specific association between serotonin transporter polymorphisms (5-Httlpr and Rs25531) and neuroticism, anxiety and depression in well-defined healthy Han Chinese. *J Affect Disord.* (2017) 207:422–8. doi: 10.1016/j.jad.2016.08.055
37. Wurtman JJ. Depression and weight gain: the serotonin connection. *J Affect Disord.* (1993) 29:183–92. doi: 10.1016/0165-0327(93)90032-F
38. Li D, Li Y. Associations of ?-carotenoid and ?-carotenoid with depressive symptoms in late midlife women. *J Affect Disord.* (2019) 256:424–30. doi: 10.1016/j.jad.2019.06.003
39. Keflie TS, Samuel A, Woldegiorgis AZ, Mihret A, Abebe M, Biesalski HK. Vitamin A and zinc deficiencies among tuberculosis patients in Ethiopia. *J Clin Tuberc Other Mycobact Dis.* (2018) 12:27–33. doi: 10.1016/j.jctube.2018.05.002
40. Ferriani LO, Silva DA, Molina M, Mill JG, Brunoni AR, da Fonseca MJM, et al. Associations of depression and intake of antioxidants and vitamin b complex: results of the Brazilian longitudinal study of adult health (Elsa-Brasil). *J Affect Disord.* (2022) 297:259–68. doi: 10.1016/j.jad.2021.10.027
41. Sowa-Kućma M, Styczeń K, Siwek M, Misztak P, Nowak RJ, Dudek D, et al. Lipid peroxidation and immune biomarkers are associated with major depression and its phenotypes, including treatment-resistant depression and melancholia. *Neurotox Res.* (2018) 33:448–60. doi: 10.1007/s12640-017-9835-5
42. Bendich A, Langseth L. Safety of Vitamin A. *Am J Clin Nutr.* (1989) 49:358–71. doi: 10.1093/ajcn/49.2.358

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REVIEW
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Fad Diets: Facts and Fiction

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The global prevalence of obesity is alarmingly high and is impacting both developed and underdeveloped countries, beyond the borders of ethnicity, sex, and age. On the other hand, the global interest in dieting has increased, and people are obsessed with certain fad diets, assuming them as a magic bullet for their long-term problems. A fad diet is a popular dietary pattern known to be a quick fix for obesity. These diets are quite appealing due to the proposed claims, but the lack of scientific evidence is a big question mark. Such diets are often marketed with specific claims that defy the basic principles of biochemistry and nutritional adequacy. These diets may have protective effects against obesity and certain chronic diseases like cardiovascular diseases, metabolic syndrome, and certain cancers. Limited evidence exists to support the proposed claims; rather certain studies suggest the negative health consequences of long-term adherence to such dietary patterns. Many fad diets have emerged in the previous few decades. This review article will explore the current evidence related to the health impacts of some most popular diets: Atkins diet, ketogenic diet, Paleolithic diet, Mediterranean diet, vegetarian diet, intermittent fasting and detox diet.

Keywords: fad diets, obesity, weight loss, metabolism, chronic disease, cardiovascular, health

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INTRODUCTION

Obesity is one of the major public health concerns in this modern era. It is now considered a global epidemic due to the gradual but continuous increase in its prevalence. The global prevalence of obesity is alarmingly high and is impacting both developed and underdeveloped countries, beyond the borders of ethnicity, sex, and age. Worldwide obesity has tripled from 1975 to 2016, while childhood obesity is increasing dramatically (1). Excessive calories from fats and sugars, large portions of food, routinely junk food intake, availability of fast foods at the doorstep and limited physical activity are some of the contributing factors to obesity (2). Obesity is an independent risk factor for morbidity and mortality. Being obese or overweight puts a person at greater risk of developing cardiovascular diseases, hypertension, insulin resistance, diabetes, reproductive issues, liver and kidney diseases (3).

Despite the growing global prevalence of obesity, there is always a group that is highly obsessed with dieting. The global interest in dieting has increased in the last two decades. A study indicated that internet searches related to weight loss queries had immensely increased between the years 2004 to 2018 (4). In the meantime, people rush toward certain fad diets (FD), assuming them as a magic bullet for their long-term problems. FD is not a scientific terminology but rather a popular or trendy dietary pattern that is known to be a quick fix for obesity (5). FD can be easily differentiated from a healthy and balanced diet based on its characteristic features: (i) promises rapid weight loss

(ii) absence of physical activity guidelines (iii) promotes short-term changes rather than achieving lifelong sustainable goals (iv) focuses on one type of food or eliminates any food group (v) cannot be maintained for life long period (vi) nutritional adequacy is questionable (vii) fails to provide health warnings for those with chronic diseases (viii) lacks scientific evidence to support the claims (5, 6) (**Figure 1**).

AIM OF THE STUDY

A wide range of FDs has been proposed to date, ranging from low carbohydrate diets to low-fat diets, high-fats to high-protein diets, those with detoxification claims, and others of the Mediterranean or Paleolithic origin. These diets are followed blindly but are associated with certain negative health outcomes as one size does not fit all. This review article will explore the current evidence related to the health impacts of some popular diets, including Atkins diet, ketogenic diet, Paleolithic diet, Mediterranean diet, vegetarian diet, intermittent fasting, and detox diet.

ATKINS DIET (AD)

In the 1970s, a low carbohydrate, high protein (LCHP) regimen was developed by cardiologist Dr. Robert Atkins, which was published in his book "Dr. Atkins' New Diet Revolution" (7). This diet was promoted as a quick weight loss plan based on a lifetime change in eating habits. Atkins believed that metabolic imbalance resulting from carbohydrate consumption is the major cause of obesity. He claimed that this is the easiest, high-energy diet that mobilizes fats more than any other diet for weight loss maintenance. The AD involves an extreme reduction of carbohydrates, i.e., less than 5% of total calorie intake, *ad libitum* intake of proteins and fats, adequate fluid intake with vitamin and mineral supplementation, and regular exercise (8).

The diet has four phases: induction phase, ongoing weight loss phase, pre-maintenance phase, and lifetime maintenance phase (**Table 1**). The modified version of the AD (MAD) is currently available with the same four phases but slightly modified net carbs consumption in each phase. The MAD is less restrictive, allowing the person to choose the number of net carbs in phase 1, i.e., 20, 40, or 100 g of carbs and fats are not just allowed but encouraged. The primary goal is not weight loss rather it has shown promising results in seizure reduction in intractable epilepsy (9–13).

Effectiveness of Atkins Diet

There is substantial evidence suggesting that AD promotes more weight loss than conventional diets. One of the first AD research was published in The New England Journal of Medicine in 2003. Brehm et al. (14) in a study allocated 53 healthy, obese women to two groups, i.e., low carbohydrate ketogenic diet (LCKD) or energy-restricted low-fat diet (LFD) (carbs: 55%, protein: 15%, fats: 30%). Over 6 months, the LCKD subjects lost 8.5 kg versus 4.2 kg in the LFD group. There were no comparable differences between the groups in serum glucose, lipids, leptin, and insulin excluding triglycerides that showed a significant reduction in the LCKD group.

In another randomized trial, 132 severely obese individuals (43% had metabolic syndrome while 39% had type 2 diabetes) were assigned to two groups. One group followed AD and the other followed LFD for 6 months. The results showed that LCD individuals lost 3.8 kg more weight than those on LFD. No significant difference was observed in both groups after 12 months (15). In another controlled trial of 1 year, 63 obese participants were randomly assigned to either the AD or conventional LFD. After 6 months, results showed that the LFD group lost less weight, i.e., 3.2 ± 5.6% than the AD group, i.e., 7.0 ± 6.5%. The AD group lost 4% more weight, had higher levels of high-density lipoprotein cholesterol (HDL-c) and lower levels of triglycerides (TG) than the other group. No significant differences between groups were noted in low-density lipoprotein cholesterol LDL-c (16).

Several meta-analyses and systemic reviews reported the promising effects of low carbohydrate diets on weight loss and cardiometabolic risk factors. Mansoor et al. (17) demonstrated that the LCD group had a significant increase in HDL-c and LDL-c, and had a greater weight loss and TG reduction in contrast to those following LFD. Hashimoto et al. (18) reported that LCD resulted in a greater reduction of body weight and body fat mass than the control diet. LCD was linked with moderately more significant advancement in weight loss and reduction of atherosclerotic cardiovascular diseases (ASCVD) risk, compared to LFD (19).

Naude et al. (20) concluded that both LCD and balanced diets had shown weight loss. After 2 years of follow-up, there was no significant difference between the diets in terms of cardiovascular and diabetes risk factors. Bueno et al. (21) found that after 12 months or more, the individuals that followed an energy-restricted very low carbohydrate diet (VLCD) (carbs: <50 g/day or 10%) compared to LFD (fats: $<30\%$) had a more significant improvement in HDL-c, LDL-c, TG and diastolic blood pressure (DBP) as well as the reduction in body weight. Hu et al. (22) compared LCD and LFD and concluded that both diets were efficient at reducing waist circumference, body weight, total cholesterol (TC), total to HDL-c ratio, LDL-c, TG, blood glucose, serum insulin, and blood pressure. LCD showed a greater decrease in TG, and less reduction in LDL-c and TC but increased HDL-c in comparison with LFD.

Health Consequences

Atkins diet has not been extensively studied while those studies that have been mentioned earlier have high dropout rates and are sometimes non-conclusive. Despite the rapid weight reduction, there are some concerns for those with comorbidities. There are some considerable potential complications associated with LCHP diets. There is conflicting evidence on the urinary stone formation tendency of LCHP diets (23). A short-term study showed that healthy subjects followed the LCHP diet for 6 weeks, decreased urine pH, increased urinary-acid excretion, and decreased calcium balance was observed in them. Therefore, they had a greater risk of stone formation (24). A prospective cohort study was conducted in Iran, involving 1,797 participants

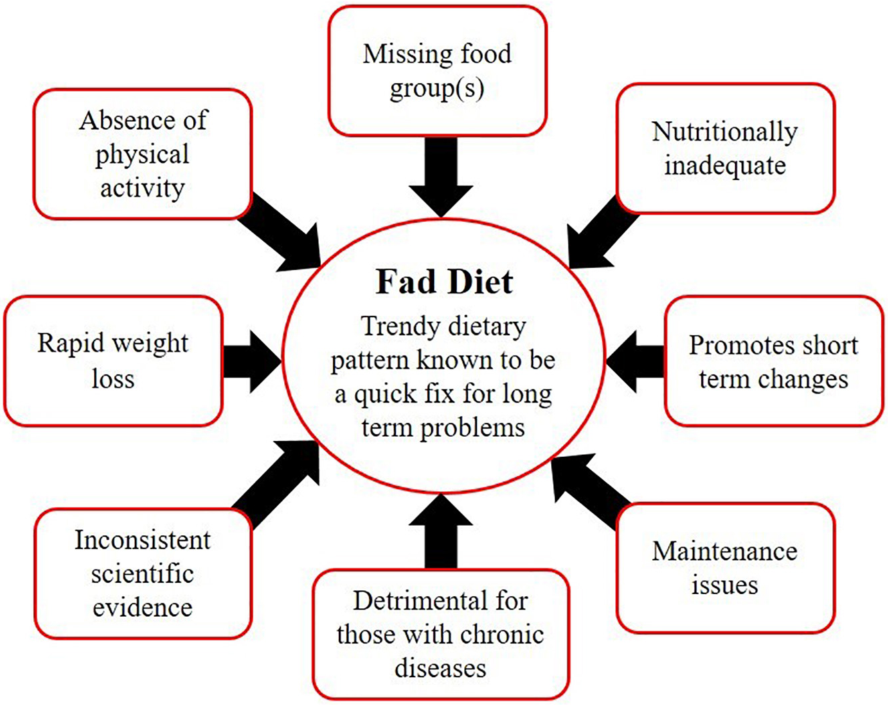

FIGURE 1 | Characteristics of fad diets.

TABLE 1 | Phases of the Atkins diet.

Phases	Duration	Major considerations	Food sources allowed	Reference
Phase 1: Induction	2 weeks	Carbs restriction to <20 g/day	Protein-rich foods: beef, poultry, fish, egg, etc., good fats: olive oil, etc.	(8)
Phase 2: Ongoing weight loss	Variable (until weight loss cease)	Gradual increase in carbs intake at a rate of 5 g per week	Nutrient dense carbs, proteins and fats	
Phase 3: Pre-maintenance	Variable (Addition phase till weight loss continues, cut back until weight loss resumes)	Additional 10 g carbs per week Cut back 5 to 10 g carbs when weight loss resumes	Nutrient dense carbs, proteins and fats	
Phase 4: Lifetime maintenance	Lifetime	Addition of a wide range of foods while keeping carbs in check, i.e., 40–90 g net carbs a day	Nutrient-dense carbs, proteins and fats	

that were followed up for almost 6 years. Results showed that a higher tertile of LCHP diet correlates with a greater risk of chronic kidney disease (CKD) (25).

Metabolic acidosis is a common complication of LCHP diets. A case of 40 years old obese woman was reported, who was presented with nausea, vomiting, dehydration, and dyspnea. Investigations revealed that she was following AD, lost 9 kg in 1 month, and laboratory findings were consistent with ketoacidosis Chen et al. (26). Pregnant and lactating mothers should be cautious when following such a diet as there is a reported case of LCD-associated ketoacidosis in a non-diabetic lactating mother (27). AD provides several benefits including weight reduction and cardio-metabolic health improvement, but limited evidence exists as compliance is the major barrier to this dietary regimen. Strict supervision by health professionals is advised as adverse metabolic sequelae can result from this type of diet.

KETOGENIC DIET (KD)

In 1923, Dr. Russell Wilder designed the classic KD for the treatment of epilepsy. The classic keto is a strict regime comprised of a 4:1 ratio, which means one part of carbs and proteins combined for four parts of fats. The use of KD for treating different diseases has increased over the past few decades. All the currently available versions are modified forms of classic KD. There are five types of KD published in the medical literature: (i) classic keto (ii) modified keto (iii) Medium-chain triglycerides oil (iv) Low glycemic index treatment (v) Modified Atkins diet.

The macronutrient ratio is the major difference between these diets. In a nutshell, KD is a VLCD that relies on a moderate amount of proteins, high fat, and low carbohydrates that provide approximately 5–10% of calories from carbohydrates, 20–25% of calories from proteins, and 65–80% of calories from fats (28). KD includes fasting, proper hydration, physical activity, and intake of electrolytes and nutritional supplements (29).

The KD works by bringing certain metabolic changes to the body. Glucose is the body's primary energy source. Carbohydrate deprivation resulting from KD causes a metabolic shift toward gluconeogenesis and ketogenesis. The preliminary shortage is managed by endogenous production of glucose from glycerol, glutamine, alanine, and lactic acid (gluconeogenesis). To keep up with the needs of the body, ketone bodies come into play and serve as an alternate energy source for the body (ketogenesis). At this stage due to low blood glucose feedback, secretion of insulin is also low, which further reduces the stimulus for fat and glucose storage. This ketotic state remains active until the body's carbohydrates needs are fulfilled (30).

Effectiveness of Ketogenic Diet

Literature is consistent with these findings that KD is an effective intervention for improving quality of life, seizure severity, and seizure frequency in epileptic patients (13, 31). KD is known for its neuroprotective action in various neurological illnesses like Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, ischemic brain injury, traumatic brain injury, depression, autism, and narcolepsy (32). In the modern era, KD is recognized as a weight loss intervention but studies suggest mixed findings. A study compared the weight loss, appetite, and hunger responses of obese men who were fed a medium carbohydrate (35%) non-ketogenic diet (MCNKD) and low carbohydrates (4%) ketogenic diet (LCKD) in a crossover manner. After 4 weeks period, significantly greater weight loss and lower *ad libitum* energy intake were observed in the LCKD group because of reduced hunger (33).

A meta-analysis concluded that KD contributes to greater long-term weight loss than LFD (21). Another study compared the impact of KD and hypocaloric diet (HCD) on metabolic parameters in obese subjects. Fifty-eight subjects followed either of the two diets for 6 months. Greater differences in fat mass, weight, waist circumference, and fasting insulin were observed in the KD group as compared to the HCD group and only KD group showed significantly increased high molecular weight (HMW) adiponectin (34). The mechanism behind successful weight loss by KD is still a scientific debate. However, certain mechanisms have been hypothesized including appetite reduction due to the action of appetite-regulating hormones, fulfilling the effect of proteins, or appetite suppressing effect of ketone bodies (33, 35–37). Weight loss can also be due to the increase in lipolysis, reduction in lipogenesis, and ease in utilizing fats due to the increased metabolic efficiency as indicated by a reduction in the respiratory quotient at rest (38–42).

In a non-randomized controlled trial, type 2 diabetes mellitus patients received either an intervention diet (KD) or served as controls. The KD group lost 10–15% of body weight, had a reduction in inflammatory markers like hsCRP, decreased WBCs, and increased TGs, HDL-c, and LDL-c (43). A recent review summarized that despite the efficacy of KD for rapid weight reduction and improved HbA1c values, KD raise LDL-c and had no superiority over other diets in terms of safety, effectivity, and sustainability (44).

In a prospective study, KD promoted negative changes in lipoprotein sub-fractions. After 6 months, KD contributed to increasing the small LDL-c and decreasing the small HDL-c, thus increasing atherogenic risk in patients (45). In another study, 12 months of KD treatment was found to be associated with decreased carotid distensibility and increased LDL-c, TC:LDL-c, and LDL-c:HDL-c ratios. No significant changes were observed in hsCRP and BMI (46). Khodabakhshi et al. (47) evaluated the effect of KD on physical activity (PA), quality of life (QOL), and biomarkers in 80 metastatic breast cancer patients. In the 12-week trial, subjects were randomly allocated to either control or KD group. No significant differences in PA and QOL scores between the groups were reported. However, the KD group showed decreases in ALP and lactate levels.

Health Consequences

Short-term minor side effects of KD are quite common, that include vomiting, nausea, gastrointestinal discomfort, fatigue, dizziness, feeling faint, decreased energy, and heartbeat alterations (48). KD initiation mostly results in hypoglycemia and lethargy (49). KD should be initiated with caution in combination with other treatments. A case report showed that the use of Valproate along with KD resulted in the development of hepatic dysfunction in a patient. The hepatotoxic effect was completely reversible as discontinuation of Valproate normalized the liver enzymes (50).

Ketogenic diet may negatively impact the lipid profile. A case report showed that following strict KD for 30–40 days, resulted in a rapid increase in LDL-c and TC. Fasting lipid profile showed HDL-c of 59 mg/dL, LDL-c of 199 mg/dL, TC of 283 mg/dL, and TG of 124 mg/dL. After discontinuation of KD and the use of statins for 4 weeks, there was a significant improvement in LDL-c (106 mg/dL) and TC (190 mg/dL). Furthermore, the patient maintained the optimal LDL-c levels after the discontinuation of statin therapy (51).

A recent case report demonstrated KD induced severe hyperlipidemia in an overweight 41 year old male. The patient had normal baseline values of lipid panel, i.e., LDL-c 99 mg/dL, HDL-c 49 mg/dL, TC 171 mg/dL, and TG 145 mg/dL. Following KD for 7 months resulted in severe hyperlipidemia as indicated by lab values, i.e., LDL-c 393 mg/dL, VLDL-c 41.5 mg/dL, HDL-c 54.4 mg/dL, TC 488.7 mg/dL, and TG 207.5 md/dL. Increasing the carbohydrates intake for 2 weeks, lipid panel showed remarkable improvement: LDL-c 279.0 mg/dL, VLDL-c 42.26 mg/dL, HDL-c 49.7 mg/dL, TC 371.2 mg/dL, and TG 211.3 mg/dL (52).

A retrospective cohort study showed that those on KD therapy had low normal bone mineral density, 8.8% of study subjects got kidney stones and 8.8% got a fracture during treatment (53). A newly recognized complication of KD is hypercalcemia. A series of case studies described the development of acute hypercalcemia about 2.1 years after initiating KD. Out of 14 patients, 13 had low levels of 1, 25-dihydroxyvitamin D, while all had low parathyroid hormone levels. Moreover, low alkaline phosphate (ALP) levels were noted in all subjects except the two oldest, while seven had impaired renal function (54).

PALEOLITHIC DIET (PD)

The PD also referred to as the Stone Age, caveman, or hunter-gatherer diet was initially introduced in 1985 by Eaton and Konner, and published by Dr. Loren Cordain in 2010 (55). It is marketed with the claims to improve health and cure diseases like obesity, cardiovascular disease, diabetes, cancer, and osteoporosis. Proponents of this dietary pattern believe that the modern diet (mainly processed foods, dairy products, grains, and legumes) is the cause of modern diseases and the obesity epidemic. Moreover, humans have evolved before agricultural development while the human diet has revolutionized more rapidly than our genetics; thus Paleolithic foods are more suited to our genetic makeup than the current modern diet (55, 56). Apart from this theory, anthropological research provides evidence that Paleolithic people used to eat a varied diet comprising of plants, grains, legumes, and game meats (57, 58).

Cordain's PD has a basic set of rules, i.e., there is no restriction on the consumption of lean meats, fruits, and non-starchy vegetables while dairy products, legumes, cereals, and processed foods are strictly restricted (**Table 2**). There is little to no focus on portions, and calories. There are three adherence levels to the PD: entry-level, maintenance level, and maximal weight loss level (**Table 3**). One has a choice not to advance to the next level if satisfied with the results of this level (55).

Effectiveness of Paleolithic Diet

Metabolic syndrome and insulin resistance are the prime focused areas in most of the literature related to PD. It does provide benefits but only to specific groups, i.e., eliminating dairy products can help people with digestive disorders. 'Liberal consumption of fruits and vegetables can have a preventive effect for inflammatory bowel diseases (IBD). At the same time, this diet being high in meat increases the risk of IBD (59).

Paleolithic diet is powerful at advancing weight reduction for the time being, even at the point when the weight reduction is unintentional (60–62). Initially, weight loss is due to the loss of water weight as this diet is low in carbohydrates. Previous studies suggest that the study participants lost 4–6% of total body weight within 10–12 weeks (63, 64). Most of the studies are based on short-term interventions and there is only one study that followed the subjects for over 2 years. In a randomized trial, 70 post-menopausal obese women were divided into the *ad libitum* PD group or Nordic Nutrition Recommendations (NNR) diet group. After 24 months, the reductions in waist circumference, fat mass, and weight were observed in both groups irrespective of the dietary regimen followed (65).

Most of the studies reported the TC reduction properties of this diet while there are mixed results for HDL-c (61, 62, 64, 66). A study was conducted to evaluate the physiological and metabolic impacts of PD in healthy adults. After 10 days of intervention, reduction in TC, LDL-c, TG, and mean arterial pressure were observed (66). In another trial, participants were randomized to PD and reference diet groups. After 2 weeks of intervention, there were greater reductions in TC, TG, and diastolic blood pressure in the PD group (61).

In another study, healthy subjects followed this dietary intervention for 10 weeks, which resulted in increased LDL-c, TC, TC:HDL-c, along with a decline in HDL-c values (64). No significant changes in fasting blood glucose were seen in most studies (65, 66). While, some studies were short-term, where HbA1c was not measured as per protocol (67). Modest reduction, i.e., 3–4 mmHg in systolic or diastolic blood pressure was reported in most studies (60, 61, 66, 67). No significant change in inflammatory markers (CRP) was reported (61, 67).

Health Consequences

The PD not only requires a big budget but is also very challenging to follow as compared to other diets (68, 69). Despite weight reduction and some favorable impact on cardiometabolic profile, this diet can have long-term consequences. Some studies suggest that this diet is not nutritionally balanced as it discourages certain food groups like whole grains, legumes, and dairy products. The

TABLE 2 | Foods in the Paleolithic diet.

Food groups	Foods allowed/restricted	Reference
Lean meat	About half of daily calories from lean animal foods are encouraged	(55)
Eggs	6–12 per week	
Fruits	All fruits are allowed Obese should be mindful of calories from high-sugar fruits	
Vegetables	All non-starchy vegetables are allowed	
Drinks and beverages	Mainly water Sugary beverages should be avoided Limited consumption of alcoholic beverages, i.e., two 4-oz servings of wine, 12-oz serving of beer or one 4-oz serving of spirits daily No tea or coffee	
Fats, oils and nuts	Unsaturated fats are allowed in moderation 4 Tbsp of oils per day 4 oz of nuts per day	
Vitamin and mineral supplements	Can be taken as per need	

TABLE 3 | Levels of Paleolithic diet.

Levels	Description	Reference
Level 1: Entry level	3 open meals*/week Addition of some transitional foods** for the sake of improving compliance	(8)
Level 2: Maintenance level	2 open meals/week No transitional foods allowed	
Level 3: Maximal weight loss level	1 open meal/week	

**Open meal; flexible meals including foods from not avoid list, intended to improve the adherence to diet **transitional foods; food items that don't meet Paleo rules.*

micronutrient deficiencies can have long-term adverse outcomes. Those who follow PD have inadequate calcium intake. A study was conducted to check the nutritional adequacy of this diet. In addition to a low intake of carbs, fats, and total calories that could have promoted weight loss, this diet provided about 50% less calcium than the daily requirement (60).

Decreased HDL-c has also been observed among healthy adults and those with comorbidities. In a study comprising 28 type 2 diabetic patients, 14 followed the PD and 10 followed the American Diabetes Association (ADA) guidelines. Results showed that there was a significant reduction in HDL-c in the PD group (62). In another study, healthy subjects followed this dietary intervention for 10 weeks, which resulted in increased LDL-c, TC, TC:HDL-c, along with a decline in HDL-c values (64). More randomized trials need to be done to highlight the consequences of such diets that eliminate one or more food groups. PD is powerful at advancing weight reduction for the time being but its efficacy in cardiovascular events is not well established as limited long-term data is available.

MEDITERRANEAN DIET (MD)

The concept of the MD emerged in the 1950s by Dr. Ancel Keys. In one of the first research that related diet and heart health, it was revealed that CVDs associated mortality rates are different in Westerns and Europeans. Lower mortality rates were observed in Europeans, even though they typically consume a moderately high-fat diet (70). Their dietary pattern can be linked to lower mortality and incidence of CVDs (71).

In 1975, Ancel Keys described this diet in his book as a complex of dietary choices followed by those living in Mediterranean regions. Whole grains, legumes, fruits, vegetables, olive oil, fish, and nuts are key components of this diet with a moderate allowance of alcohol, dairy products, and meat [Keys, (72)]. Traditionally, this diet derives its most calories from fish and plant-based foods. Fats account for 30% of calories which are mostly polyunsaturated fatty acid (PUFA) and monounsaturated fatty acids (MUFA), while carbohydrates provide 50–55% of calories from low glycemic index carbohydrates and proteins provide 15–20% of calories (73).

Effectiveness of Mediterranean Diet

Mediterranean diet is the most extensively studied diet to date. In a previous review, it has been summarized that MD is nutritionally adequate for the general public and may have the potential of preventing micronutrient deficiencies (74). Research shows that it has preventive and therapeutic potential for many chronic diseases like non-alcoholic fatty live disease (NAFLD), CVDs, metabolic syndrome, and certain cancers like colorectal and breast cancer (75). In a 2-year trial, weight loss by LFD, AD, and MD was compared and results showed that the AD group had the highest mean weight loss, i.e., – 4.7 ± 6.5 kg, while the MD group stood second with a mean weight loss of – 4.4 ± 6 kg and LFD group lost –2.9 ± 4.2 kg. Following changes were recorded in the MD group: increased molecular adiponectin reduced serum leptin, and CRP levels (76).

In another controlled trial, 259 subjects were randomly allocated to American Diabetic Association (ADA) diet, traditional MD, or low carbohydrate Mediterranean diet (LCM) group. After 12 months, the LCM group had the highest weight reduction, increased HDL-c, improved LDL-c, TG and HbA1c (77). Another study described the effectiveness of MD in the primary prevention of CVDs [Estruch et al. (78)]. A study investigated the protective effect of MD against cancer and found that greater compliance with MD patterns reduces the risk of non-tobacco linked cancers in both men and women (79).

Most MD studies are short-duration studies, only a few studies focused on the long-term impacts of following MD. In a study, non-diabetic elderly subjects (n = 3,541) at higher risk of CVD were randomized to three intervention groups: control diet, MD with nuts or MD with extra virgin olive oil. New cases of diabetes were recorded after regular intervals (median follow-up duration = 4.1 years) and results showed that MD with extra virgin olive oil was associated with a reduced risk of diabetes (80). In another 5 years clinical trial, subjects (n = 7,447) who were type 2 diabetics or those with risk factors of CVDs, were randomized to three intervention groups: control diet, MD with nuts, or MD with extra virgin olive oil. After the specified period, there was no significant weight reduction in all the groups, while the MD group had a significant reduction in central obesity [Estruch et al. (81)].

Health Consequences

No evidence of adverse effects associated with MD is available in the literature. Rather, MD has preventive and therapeutic potential for many chronic diseases. It is highly suitable for the general public for the prevention of micronutrient deficiencies and specifically for those patients who are more health-conscious than just weight loss oriented.

VEGETARIAN DIET (VD)

The VD is a dietary pattern characterized by no consumption of meat and meat products, seafood, poultry, and sometimes other animal products like eggs, animal milk, and honey. Some studies have linked meat intake with an increased risk of chronic diseases, while others indicate a positive association between low meat intake and life expectancy (82, 83). VD are of four main types: (i) a lacto-ovo-vegetarian does not consume any meat product but consumes eggs and dairy products (ii) a lactovegetarian does take dairy products but does not consume eggs and meat products (iii) an ovo-vegetarian does not eat meat products and dairy products and are free to consume eggs (iv) a vegan does not consume any animal products, including meat, eggs, dairy products, and honey (84). Vegan diets include different subtypes: raw vegan, vegan (general), and whole-food vegan (**Figure 2**). Each subtype has its own set of foods allowed and restricted with one thing in common, i.e., meat products restriction. This dietary pattern is gaining much popularity in the general population, especially in the Western world (85). There are various reasons for adopting this dietary profile, including religious beliefs, ethical motivation, cultural aspects, and health considerations (85, 86).

FIGURE 2 | Foods allowed and restricted in vegan diets.

Effectiveness of Vegetarian Diet

Several epidemiological studies reported a lower cardiometabolic risk in the vegan population. A study concluded that non-vegetarians have a higher type 2 diabetes prevalence (7.6%) than vegetarians (2.9%). While, the prevalence rate also varies with the type of VD, i.e., 3.2% in lacto-ovo vegetarians, 4.8% in pesco-vegetarians and 6.1% in semi-vegetarians. This can be explained by the low-glycemic-response associated with these diets as vegetarian diets typically include foods that have a low glycemic index such as beans, legumes, nuts, some fruits and vegetables (87). Glycemic control via a VD is quite controversial, as these are high carbohydrate diets. Some studies have shown that vegetarians also have increased life expectancy (82). Generally, vegetarians are more health-conscious and have lower BMI than the general population (88). The Seventh Day Adventist study showed a lower mean BMI, i.e., 23.6 kg/m^2 in the vegan population (89). In a 5-year prospective study, 22,000 subjects having different dietary patterns were checked for their weight gain during this period. Vegans had the lowest weight gain as compared to meat-eaters and fish eaters (90).

Red meat and poultry intake were most strongly linked to increased risk of esophageal adenocarcinoma and gastric cardia or non-cardia adenocarcinoma, respectively (91). On the other hand, lower rates of heart diseases and cancers have been observed in vegetarians in comparison with those following other dietary patterns (92, 93). A better cardiometabolic risk profile is generally present in vegetarians, i.e., lower BMI, TC, and LDL-c [Chen et al. (94); De Biase et al. (95)]. A cross-sectional study investigated the lipid profile of fish-eaters, meat-eaters, and vegetarians. Not only the vegans have a lower BMI but also favorable serum lipid levels: lower LDL-c, TC, and apolipoproteins (96).

In a study, out of 26,346 participants, 1,079 cases of prostate cancer were identified and results showed the protective effect of vegan diets against prostate cancer in the white population (97). This protective effect against prostate cancer may be due to the higher fiber intake. Some other studies are either short-term or have a very small sample size, showing mixed findings related to colorectal cancer and breast cancer. In a study, 2,304 patients from 10 European countries were assessed for their dietary intake to find the impact of diet on the risk of cancers. Not poultry but red meat intake was found to be associated with an increased risk of esophageal cancer and upper aerodigestive tract (UADT) cancer. Furthermore, vegetable and fruit intake are significantly associated with a reduced risk of UADT cancer (98).

Butler et al. (99) demonstrated that the higher the intake of vegetable-fruit -soy dietary pattern, the lower the breast cancer hazard ratio among postmenopausal women. Another study showed a significant association between consumption of vegetables and risk of esophageal adenocarcinoma. Elimination of potentially harmful dietary components like animal protein, saturated fats, and cholesterol can be the reason for these benefits. These benefits can also be due to the addition of dietary fiber, phytochemicals, and antioxidants rich in beneficial dietary components like whole grains, legumes, nuts, fruits, and vegetables (91).

Health Consequences

This diet is associated with fluctuations in micronutrients intake because of the day-to-day variation in the menu. Depending upon the type of VD, vegetarians are potentially at increased risk of micronutrient deficiencies such as calcium, zinc, iron, vitamin E, vitamin B12, essential fatty acids, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) (100). A study reported that half of the vegan participants were micronutrient deficient as compared to omnivores (101). Vegetarians have lower serum vitamin B12 levels as plant sources are deficient in this vitamin (101, 102). As VD is generally low in calcium due to the suboptimal intake of dairy, vegetarians are at greater risk of bone fractures due to the lower bone mineral density (103).

Thus supplementation of certain vitamins like vitamin B12 and vitamin D is needed to avoid these deficiencies among vegetarians (84). Vitamin B12 supplements are especially important for vegan pregnant and lactating mothers as a preventive therapy for deficiency in their babies (104). VD can be nutritionally adequate, so it may be helpful in chronic disease prevention and treatment. Benefits and harms depend upon the dietary choices so the individualized plan fulfilling the micronutrient requirements must be carefully developed by a professional.

INTERMITTENT FASTING (IF)

The IF is gaining much popularity and is widely adopted as an effective weight loss intervention. Contrary to the conventional weight loss programs that are based on calorie restriction, IF is more about scheduled eating. Some of the key features of IF are abstinence from food for a certain period, followed by a period of normal eating. There are various versions of IF but the most popular of these are alternate day fasting (ADF), 5:2 diet or periodic fasting (PF), and time-restricted feeding (TRF). The frequency and duration of fast cycles may differ among all types (**Table 4**).

Effectiveness of Intermittent Fasting

The alternate-day fasting (ADF) approach has been tested for its metabolic effects. In a study, healthy young men (n = 8) were subjected to ADF for 20 h/day for 15 days. After the specified study period, weight remained unchanged (86.4 ± 2.3 kg) while the increase in glucose uptake, i.e., 7.3 ± 0.3 mg/kg/min that was previously 6.3 ± 0.6 mg/kg/min, and prominent increase in lipolysis of adipose tissues were observed (106). Another study showed that when non-obese subjects (8 women and 8 men) fasted for 22 days on alternate days, they lost 4 ± 1% of their initial fat mass and 2.5 ± 0.5% of their initial body weight. However, a decrease in fasting insulin and non-significant change in glucose and ghrelin were also reported (107).

A randomized crossover trial was conducted to evaluate the fasting-induced acute changes in biomarkers. Healthy volunteers (n = 30) were randomized into two groups: (i) normal eating for 28 ± 4 h then water-only fasting for 28 ± 4 h (ii) 28 ± 4 h of water-only fasting then 28 ± 4 h of normal eating. Blood samples were drawn and analyzed at baseline, day 1 and day 2. Laboratory findings suggested that the fasting intervention acutely increased hemoglobin, hematocrit, red blood cell count, human growth hormone, and HDL-c; on the other hand, decreased body weight, bicarbonates, and TGs, as compared to the normal eating day. Moreover, cholesterol and human growth hormone returned to baseline after 48 h (108).

Night-time fasting (NTF) has been linked to lower energy intake, consequently resulting in weight loss. In a study, twenty-nine healthy young men were subjected to 9 h of NTF for 2 weeks, then 1 week washout period followed by 2 weeks of controlled conditions. Results showed that the participants had less total calorie intake in the NTF phase as compared to controlled conditions. Significant differences in weight change were also reported, i.e., – 0.4 kg for NTF and + 0.6 kg for control (109).

In a randomized trial of 3 months, young overweight premenopausal women (n = 107) were randomly assigned to two groups: two consecutive days of fasting (25% energy restriction)/week or fasting for all days of the week. Both interventions were found to be equally good at a weight and showed improvement in risk markers of CVDs, cancer, and diabetes for example reduction in leptin, leptin to adiponectin ratio, inflammatory markers, fasting insulin, insulin resistance, blood pressure, and lipids (110).

Fasting also impacts the appetite by influencing the appetite-regulating hormones (110, 111). A previous systematic review summarized that IF may have the potential to provide metabolic benefits in terms of improving insulin resistance, thus providing better glycemic control as IF showed a significant decline in fasting glucose levels as compared to controls. Moreover, IF was associated with a decline in BMI, fat mass, and leptin while an increase in adiponectin (112). Headland et al. (113) evaluated the effectiveness of intermittent energy restriction (IER) in improving weight and biological markers in long-term studies. Irrespective of duration, IER was associated with weight loss.

TABLE 4 | Types of intermittent fasting.

Types	Description	Fasting definition	Normal eating	Reference
Alternate day fasting	Fasting alternated with a day of normal eating	0–25% of TCN*	*ad libitum*	(105)
5:2 diet or periodic fasting	Fasting for 2 days with normal eating for 5 days	0–25% of TCN*	*ad libitum*	
Time-restricted feeding	Normal eating within a window of < 8 h per day	–	*ad libitum*	

**TCN, total caloric needs.*

TABLE 5 | Commercial detox diets.

Diet type	Duration	Foods allowed	Proposed claims	References
Liver cleansing diet	8 weeks	Plant-based, dairy-free, low fat, high fiber, unprocessed foods are allowed. Epsom salt and liver tonics are also consumed.	Improved energy levels and liver function Toxins removal Improved immune response Efficient metabolism of fats and better weight control	(117)
Lemon detox diet/ Master cleanser	10 days	A liquid only diet based on purified water, lemon juice, tree syrup and cayenne pepper. A mild laxative herbal tea and sea salt water is also incorporated.	Toxins removal Shiny hair, glowing skin and strong nails Weight loss	(118)
The clean cleanse	21 days	Breakfast and dinner comprise probiotic capsules, cleanse supplements and cleanse shakes. A solid meal in lunch while avoiding gluten, dairy, corn, soy, pork, beef, refined sugars, some fruits and vegetables.	Toxins removal Improved energy, digestion, sleep and mental health Reduction in joint pains, headaches, constipation and bloating	(119)
Martha's vineyard detox diet	21 days	Herbal teas, vegetable soups and juices, specially formulated tablets, powders and digestive enzymes are on the menu.	Weight loss up to 9.5 kg Toxins removal Improved energy levels	(120)
Weekend wonder detox	48 h	Protein-rich meals salads, detox-promoting super foods and beverages. Healthy lifestyle, spa treatments and herbal remedies.	Toxins removal Improved organs' function Strengthen body Enhance beauty	(121)
Fat flush	2 weeks	Large meals are replaced with dilute cranberries, hot water with lemon, pre-prepared cocktails, supplements and small meals	Toxins removal Reduced stress Weight loss Improved liver function	(122)
Blue print cleanse	3 days	Consumption of six pre-prepared vegetable and fruit juices is allowed per day.	Toxins removal	(123)
The Hubbard purification rundown	Several weeks	Niacin doses along with sustained consumption of vitamin-A, B, C, D, and E. Daily exercise with balanced meals. Restriction of alcohol and drugs. Sitting in a sauna for ≤ 5 h each day.	Toxins removal from fat stores Improved memory and intelligence quotient Better blood pressure and cholesterol levels	(124)

However, IER was not found to be superior to continuous energy restriction (CER) in terms of weight loss, blood lipids, glucose, and insulin levels.

Health Consequences

Some short-term studies highlighted the potential harms posed by IF among normal-weight subjects. IF induces lipolysis, resulting in increased free fatty acids (FFA). So whether it be ADF, periodic fasting, or else, a prolonged course of fasting can lead to large fluctuations in FFA in normal-weight individuals. A study showed that these fluctuations were three times greater than those typically seen after an overnight fast. Furthermore, it induced reductions in insulin sensitivity and acute glucose-simulated insulin response (114). Despite the effectiveness of IF in weight loss as indicated by several studies, the current evidence is non-conclusive. The prime focus of available literature is weight loss but little is known about its sustainability and long-term health effects. More long-term trials should be conducted to draw a clear conclusion.

DETOX DIETS (DD)

The popularity of detoxification dates back to Greek, Roman, Indian, and Native American cultures. Many effective approaches that are still used for the removal of toxins include fasting, saunas, herbs, rebounding, dry brush, water, rest, exercise, and meditation (115). However, detoxification or DD are interventional diets specifically designed for toxins elimination, health promotion, and weight management. These short-term dietary interventions involve multiple approaches, including total calorie restriction, dietary modification, or juice fasts, and often involve the use of additional minerals, vitamins, diuretics, laxatives, or cleansing foods. Some commercial DDs have been listed in **Table 5**. These are most commonly prescribed by naturopathic doctors to prevent or treat a number of conditions like gastrointestinal disorders, inflammation, autoimmune disorders, chronic fatigue syndrome, fibromyalgia, and weight loss (116).

The DDs have not been extensively investigated; however, the handful of available studies have methodological limitations like sampling bias, small sample sizes, relying on self-reporting, and absence of control groups. Despite the emerging popularity, these diets fail to identify the mechanisms of eliminating toxins or even the specific toxins removed by a particular diet. Detox approaches defy the general principles of human physiology as the liver and kidneys are quite efficient in removing both exogenous and endogenous toxins from our body, along with extra-renal excretion of toxins in sebum and sweat (125).

Effectiveness of Detox Diets

Currently, there is no clinical evidence confirming or negating the effectiveness of commercially available detox regimes for losing weight. Because of its emerging popularity, this area needs attention. So, in the absence of scientific evidence, results can be extrapolated from other closely related studies. It is known that the success rate of dieting, in general, is only 20% (126). This may be possible because humans and animals have natural mechanisms to counter the weight loss as starvation can have negative health consequences like reduced fertility and even death. Calorie restriction alters the neuropeptides' expression in the hypothalamus; which reduces metabolic rate and stimulates appetite, resulting in a weight loss plateau (127).

Furthermore, studies in mice have shown binge eating followed by a period of energy restriction, though this phenomenon is not established in humans yet (128). A study conducted by Mazurak et al. (129) showed that fasting raised cortisol levels in young healthy women. Another study reported an increase in stress hormone levels in females due to the restricted intake of 1,200 kcal/day (130). There is considerable evidence that stress stimulates appetite, thus promoting weight gain via elevations of cortisol (131).

Many of the DD are liquid-based, low-calorie, and nutrient-poor. For example, a part of BluePrint Cleanse, Excavation Cleanse, provides only 19 g protein and 860 kcal/day which is far below the actual requirement. Food and Agriculture Organization (FAO) recommends a minimum of 0.83 g/kg body weight of high-quality protein and 1,680 kcal/day for an adult (132, 133). Based on the previous work, DD may induce stress, raise cortisol levels and increase appetite, resulting in difficulty in losing weight, followed by binge eating and weight gain (128–130).

It is quite alarming that the components of detox products may not be according to the labels as there is no regulatory authority that approves such products. A case was reported in Spain that a 50 year old man with no history of relevant medical illness, presented with diffuse abdominal pain, lethargy, profuse diarrhea, and vomiting after ingesting Epsom salt during a liver cleansing diet. That person died within 72 h from the onset of symptoms. Forensic and clinical investigations concluded that instead of magnesium sulfate heptahydrate, the supplier had mistakenly added hydrated manganese sulfate resulted in manganese intoxication (134).

Energy-restricted DDs are capable of short-term weight loss. But still, there is a high likelihood of health risks from detox products because of their nutritional inadequacy. As no convincing evidence exists in this domain so such diets and products need to be discouraged by health professionals and must be subjected to regulatory review and monitoring.

CONCLUSION

Fad diets facilitate fast and easy weight loss, improve appearance, and do not require a longer time to achieve the results. These diets are effective in improving health to some extent. However, compliance is always a significant concern because of the unrealistic combinations and nutritional inadequacy due to the complete elimination of one or more essential food groups. Despite the rapid weight reduction, there are some concerns for those with comorbidities. All these diets have not been extensively studied while those studies that have been mentioned in the literature have high dropout rates and are sometimes non-conclusive. More randomized controlled trials of prolonged duration need to be done to establish the safety of FDs for the public and to make people aware of the possible consequences of long-term adherence to such dietary patterns.

AUTHOR CONTRIBUTIONS

AT and AR: conceptualization. AT: writing – original draft preparation. AT, AN, RR, AR, RA, CS, and CM: writing – review and editing. AR and RA: supervision. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. World Health Organization [WHO]. *WHO Obesity and Overweight*. (2021). Available online at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight/ (Accessed September 12, 2021).
2. Wright SM, Aronne LJ. Causes of obesity. *Abdom Imaging.* (2012) 37:730–2. doi: 10.1007/s00261-012-9862-x
3. Uzogara SG. Obesity epidemic, medical and quality of life consequences: a review. *Int J Public Health Res.* (2017) 5:1–12.
4. Teng Y, Huang S, Li Z, Xie Q, Zhang M, Lou Q, et al. Seasonal variation and trends in the internet searches for losing weight: an infodemiological study. *Obes Res Clin Pract.* (2020) 14:225–33. doi: 10.1016/j.orcp.2020.04.001
5. Gui G. Fad diets, fats & weight management. *Singapore Fam Physician.* (2008) 34:14–9.

6. Bastin S. *Fad Diets*. Lexington, KY: University of Kentucky (2004).
7. Atkins RC. *Dr. Atkins' Diet Revolution: The High Calorie Way to Stay Thin Forever*. New York, NY: McKay (1973).
8. Apovian C, Brouillard E, Young L. *Clinical Guide to Popular Diets*. New York: CRC Press (2018).
9. Chen W, Kossoff EH. Long-term follow-up of children treated with the modified atkins diet. *J Child Neurol.* (2012) 27:754–8. doi: 10.1177/0883073812441062
10. Kang H, Lee HS, You SJ, Kang DC, Ko T, Kim HD. Use of a modified atkins diet in intractable childhood epilepsy. *Epilepsia.* (2007) 48:182–6. doi: 10.1111/j.1528-1167.2006.00910.x
11. Kossoff EH, Turner Z, Bluml RM, Pyzik PL, Vining EPGA. Randomized, crossover comparison of daily carbohydrate limits using the modified atkins diet. *Epilepsy Behav.* (2007) 10:432–6. doi: 10.1016/j.yebeh.2007.01.012
12. Kossoff EH, Rowley H, Sinha SR, Vining EPGA. Prospective study of the modified atkins diet for intractable epilepsy in adults. *Epilepsia.* (2008) 49:316–9. doi: 10.1111/j.1528-1167.2007.01256.x
13. Kverneland M, Molteberg E, Iversen PO, Veierød MB, Taubøll E, Selmer KK, et al. Effect of modified atkins diet in adults with drug-resistant focal epilepsy: a randomized clinical trial. *Epilepsia.* (2018) 59:1567–76. doi: 10.1111/epi.14457
14. Brehm BJ, Seeley RJ, Daniels SR, D'alessio DAA. Randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *J Clin Endocrinol Metab.* (2003) 88:1617–23. doi: 10.1210/jc.2002-021480
15. Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, et al. Low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med.* (2003) 348:2074–81. doi: 10.1056/NEJMoa022637
16. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, et al. Randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med.* (2003) 348:2082–90. doi: 10.1056/NEJMoa022207
17. Mansoor N, Vinknes KJ, Veierød MB, Retterstøl K. Effects of low-carbohydrate diets v. low-fat diets on body weight and cardiovascular risk factors: a meta-analysis of randomised controlled trials. *Br J Nutr.* (2016) 115:466–79. doi: 10.1017/S0007114515004699
18. Hashimoto Y, Fukuda T, Oyabu C, Tanaka M, Asano M, Yamazaki M, et al. Impact of low-carbohydrate diet on body composition: meta-analysis of randomized controlled studies. *Obes Rev.* (2016) 17:499–509. doi: 10.1111/obr.12405
19. Sackner-bernstein J, Kanter D, Kaul S. Dietary intervention for overweight and obese adults: comparison of low-carbohydrate and low-fat diets. A meta-analysis. *PLoS One.* (2015) 10:e0139817. doi: 10.1371/journal.pone.0139817
20. Naude CE, Schoonees A, Senekal M, Young T, Garner P, Volmink J. Low carbohydrate versus isoenergetic balanced diets for reducing weight and cardiovascular risk: a systematic review and meta-analysis. *PLoS One.* (2014) 9:e100552. doi: 10.1371/journal.pone.0100652
21. Bueno NB, de Melo SIV, De Oliveira SL, da Rocha Ataide T. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *Br J Nutr.* (2013) 110:1178–87. doi: 10.1017/S0007114513000548
22. Hu T, Mills KT, Yao L, Demanelis K, Eloustaz M, Yancy WS, et al. Systematic reviews and meta- and pooled analyses effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: a meta-analysis of randomized controlled clinical trials. *Am J Epidemiol.* (2012) 176:S44–54. doi: 10.1093/aje/kws264
23. Nouvenne A, Ticinesi A, Morelli I, Guida L, Borghi L, Meschi T. Fad diets and their effect on urinary stone formation. *Transl Androl Urol.* (2014) 3:303–12. doi: 10.3978/j.issn.2223-4683.2014.06.01
24. Reddy ST, Wang C, Sakhaee K, Brinkley L, Pak CYC. Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism. *Am J Kidney Dis.* (2002) 40:265–74. doi: 10.1053/ajkd.2002.34504
25. Farhadnejad H, Asghari G, Emamat H, Mirmiran P, Azizi F. Low-carbohydrate high-protein diet is associated with increased risk of incident chronic kidney diseases among Tehranian adults. *J Ren Nutr.* (2018) 29:343–9. doi: 10.1053/j.jrn.2018.10.007
26. Chen T, Smith W, Rosenstock JL, Lessnau KA. Life-threatening complication of atkins diet. *Lancet.* (2006) 367:958. doi: 10.1016/S0140-6736(06)68394-3
27. von Geijer L, Ekelund M. Ketoacidosis associated with low-carbohydrate diet in a non-diabetic lactating woman: a case report. *J Med Case Rep.* (2015) 9:224–6. doi: 10.1186/s13256-015-0709-2
28. Gershuni VM, Yan SL, Medici V. Nutritional ketosis for weight management and reversal of metabolic syndrome. *Curr Nutr Rep.* (2018) 7:97–106. doi: 10.1007/s13668-018-0235-0
29. Neal EG, Zupec-kania B, Pfeifer HH. Carnitine, nutritional supplementation and discontinuation of ketogenic diet therapies. *Epilepsy Res.* (2012) 100:267–71. doi: 10.1016/j.eplepsyres.2012.04.021
30. Dąbek A, Wojtala M, Pirola L, Balcerczyk A. Modulation of cellular biochemistry, epigenetics and metabolomics by ketone bodies. implications of the ketogenic diet in the physiology of the organism and pathological states. *Nutrients.* (2020) 12:788–801. doi: 10.3390/nu12030788
31. Roehl K, Falco-walter J, Ouyang B, Balabanov A. Modified ketogenic diets in adults with refractory epilepsy: efficacious improvements in seizure frequency, seizure severity, and quality of life. *Epilepsy Behav.* (2019) 93:113–8. doi: 10.1016/j.yebeh.2018.12.010
32. Stafstrom CE, Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol.* (2012) 3:59. doi: 10.3389/fphar.2012.00059
33. Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. *Am J Clin Nutr.* (2008) 87:44–55. doi: 10.1093/ajcn/87.1.44
34. Partsalaki I, Karvela A, Spiliotis BE. Metabolic impact of a ketogenic diet compared to a hypocaloric diet in obese children and adolescents. *J Pediatr Endocrinol Metab.* (2012) 25:697–704. doi: 10.1515/jpem-2012-0131
35. Veldhorst M, Smeets A, Soenen S, Hochstenbach-Waelen A, Hursel R, Diepvens K, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav.* (2008) 94:300–7. doi: 10.1016/j.physbeh.2008.01.003
36. Westerterp-Plantenga MS, Nieuwenhuizen A, Tome D, Soenen S, Westerterp KR. Dietary protein, weight loss, and weight maintenance. *Annu Rev Nutr.* (2009) 29:21–41. doi: 10.1146/annurev-nutr-080508-141056
37. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Ketosis and appetite-mediating nutrients and hormones after weight loss. *Eur J Clin Nutr.* (2013) 67:759–64. doi: 10.1038/ejcn.2013.90
38. Cahill GF. Fuel metabolism in starvation. *Annu RevNutr.* (2006) 26:1–22. doi: 10.1146/annurev.nutr.26.061505.111258
39. Veldhorst MA, Westerterp-plantenga MS, Westerterp KR. Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am J Clin Nutr.* (2009) 90:519–26. doi: 10.3945/ajcn.2009.27834.1
40. Paoli A, Cenci L, Fancelli M, Parmagnani A, Fratter A, Cucchi A, et al. Ketogenic diet and phytoextracts comparison of the efficacy of mediterranean, zone and Tisanoreica diet on some health risk factors. *Agro Food Ind Hi Tech.* (2010) 21:24–9.
41. Paoli A, Grimaldi K, Bianco A, Lodi A, Cenci L, Parmagnani A. Medium term effects of a ketogenic diet and a mediterranean diet on resting energy expenditure and respiratory ratio. *BMC Proc.* (2012) 6:37. doi: 10.1186/1753-6561-6-S3-P37
42. Tagliabue A, Bertoli S, Trentani C, Borrelli P, Veggiotti P. Effects of the ketogenic diet on nutritional status, resting energy expenditure, and substrate oxidation in patients with medically refractory epilepsy: a 6-month prospective observational study. *Clin Nutr.* (2012) 31:246–9. doi: 10.1016/j.clnu.2011.09.012
43. Hallberg SJ, Mckenzie AL, Williams PT, Bhanpuri NH, Peters AL, Campbell WW, et al. Effectiveness and safety of a novel care model for the management of type 2 diabetes at 1 year: an open-label, non-randomized, controlled study. *Diabetes Ther.* (2018) 9:583–612. doi: 10.1007/s13300-018-0373-9
44. O'Neill B, Raggi P. The ketogenic diet: pros and cons. *Atherosclerosis.* (2020) 292:119–26. doi: 10.1016/j.atherosclerosis.2019.11.021
45. Azevedo de Lima P, Baldini Prudêncio M, Murakami DK, Pereira de Brito Sampaio L, Figueiredo Neto AM, Teixeira Damasceno NR. Effect of classic ketogenic diet treatment on lipoprotein subfractions in children and adolescents with refractory epilepsy. *Nutrition.* (2017) 33:271–7. doi: 10.1016/j.nut.2016.06.016

46. Kapetanakis M, Liuba P, Odermarsky M, Lundgren J, Hallbo T. Effects of Ketogenic Diet on Vascular Function. *Eur J Paediatr Neurol.* (2014) 18:489–94. doi: 10.1016/j.ejpn.2014.03.006
47. Khodabakhshi A, Seyfried TN, Kalamian M, Beheshti M, Davoodi SH. Does a ketogenic diet have beneficial effects on quality of life, physical activity or biomarkers in patients with breast cancer: a randomized controlled. *Nutr J.* (2020) 19:87–96. doi: 10.21203/rs.3.rs-19350/v2
48. Bostock ECS, Kirkby KC, Taylor BV, Hawrelak JA. Consumer reports of "Keto Flu" associated with the ketogenic diet. *Front Nutr.* (2020) 7:20. doi: 10.3389/fnut.2020.00020
49. Lin A, Turner Z, Doerrer SC, Stanfield A, Kossoff EH. Complications during ketogenic diet initiation: prevalence, treatment and influence on seizure outcomes. *Pediatr Neurol.* (2017) 68:35–9. doi: 10.1016/j.pediatrneurol.2017.01.007
50. Stevens CE, Turner Z, Kossoff EH. Hepatic dysfunction as a complication of combined valproate and ketogenic diet. *Pediatr Neurol.* (2016) 54:82–4. doi: 10.1016/j.pediatrneurol.2015.10.006
51. Noain JS, Minupuri A, Kulkarni A, Zheng S. Significant impact of the ketogenic diet on low-density lipoprotein cholesterol levels. *Cureus.* (2020) 12:10–3. doi: 10.7759/cureus.9418
52. Swaid B. Severe hyperlipidemia with LDL cholesterol of 393 Milligrams per decilitre after 7 months of high fat ketogenic diet: a rare case report. *J Endocr Soc.* (2021) 5:A37–8. doi: 10.1210/jendso/bvab048
53. Draaisma JMT, Hampsink BM, Janssen M, van Houdt NBM, Linders ETAM, Willemsen MA. The ketogenic diet and its effect on bone mineral density: a retrospective observational cohort study. *Neuropediatrics.* (2019) 50:353–8. doi: 10.1055/s-0039-1693059
54. Hawkes CP, Roy SM, Dekelbab B, Frazier B, Grover M, Haidet J, et al. hypercalcemia in children using the ketogenic diet: a multicenter study. *J Clin Endocrinol Metab.* (2021) 106:e485–95. doi: 10.1210/clinem/dgaa759
55. Cordain L. *The Paelo Diet*. Hoboken: John Wiley & Sons (2010).
56. Eaton SB, Konner MJ, Cordain L. Diet-dependent acid load, paleolithic nutrition, and evolutionary health promotion. *Am J Clin Nutr.* (2010) 91:295–7. doi: 10.3945/ajcn.2009.29058.Am
57. Katz SH, Weaver WW. *Game*. New York: Scribner (2003).
58. Eisenstein M. Evolution: the first supper. *Nature.* (2010) 468:S8–9. doi: 10.1038/468S8a
59. Hou JK, Abraham B, El-serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol.* (2011) 106:563–73. doi: 10.1038/ajg.2011.44
60. Osterdahl M, Kocturk T, Koochek A, Wandell PE. Effects of a short-term intervention with a paleolithic diet in healthy volunteers. *Eur J Clin Nutr.* (2008) 62:682–5. doi: 10.1038/sj.ejcn.1602790
61. Boers I, Muskiet FAJ, Berkelaar E, Schut E, Penders R, Hoenderdos K, et al. Favourable effects of consuming a palaeolithic-type diet on characteristics of the metabolic syndrome: a randomized controlled pilot-study. *Lipids Health Dis.* (2014) 13:160–72. doi: 10.1186/1476-511X-13-160
62. Masharani U, Sherchan P, Schloetter M, Stratford S, Xiao A, Sebastian A, et al. Metabolic and physiologic effects from consuming a hunter-gatherer (paleolithic)-type diet in type 2 diabetes. *Eur J Clin Nutr.* (2015) 69:944–8. doi: 10.1038/ejcn.2015.39
63. Lindeberg S, Jönsson T, Granfeldt Y, Borgstrand E, Soffman J, Sjöström K, et al. Palaeolithic diet improves glucose tolerance more than a mediterranean-like diet in individuals with ischaemic heart disease. *Diabetologia.* (2007) 50:1795–807. doi: 10.1007/s00125-007-0716-y
64. Smith MM, Trexler ET, Sommer AJ, Starkoff BE, Devor ST. Unrestricted paleolithic diet is associated with unfavorable changes to blood lipids in healthy subjects. *Int J Exerc Sci.* (2014) 7:128–39.
65. Mellberg C, Sandberg S, Ryberg M, Eriksson M, Brage S, Larsson C, et al. Long-term effects of a palaeolithic-type diet in obese postmenopausal women: a 2-year randomized trial. *Eur J Clin Nutr.* (2014) 68:350–7. doi: 10.1038/ejcn.2013.290
66. Frassetto LA, Schloetter M, Mietus-Synder M, Morris RC, Sebastian A. Metabolic and Physiologic Improvements from Consuming a Paleolithic, Hunter-Gatherer Type Diet. *Eur J Clin Nutr.* (2009) 63:947–55. doi: 10.1038/ejcn.2009.4
67. Jönsson T, Granfeldt Y, Ahrén B, Branell U-C, Pålsson G, Hansson A, et al. Beneficial effects of a paleolithic diet on cardiovascular risk factors in type 2 diabetes: a randomized cross-over pilot study. *Cardiovasc Diabetol.* (2009) 8:35–48. doi: 10.1186/1475-2840-8-35
68. Jönsson T, Granfeldt Y, Lindeberg S, Hallberg A. Subjective satiety and other experiences of a paleolithic diet compared to a diabetes diet in patients with Type 2 diabetes. *Nutr J.* (2013) 12:105–11. doi: 10.1186/1475-2891-12-105
69. Manheimer EW, Van Zuuren EJ, Fedorowicz Z, Pijl H. Paleolithic nutrition for metabolic syndrome: systematic review and meta-analysis. *Am J Clin Nutr.* (2015) 102:922–32. doi: 10.3945/ajcn.115.113613.1
70. Keys A, Grande F. Role of dietary fat in human nutrition. *Am J Public Health.* (1957) 47:1520–30. doi: 10.2105/ajph.47.12.1530
71. Martınez-Gonzalez MA, Sanchez-Villegas A. The emerging role of mediterranean diets in cardiovascular epidemiology: monounsaturated fats, olive oil, red wine or the whole pattern? *Eur J Epidemiol.* (2004) 19:9–13. doi: 10.1023/b:ejep.0000013351.60227.7b
72. Keys AB. *How to Eat Well and Stay Well the Mediterranean Way*. New York, NY: Doubleday (1975).
73. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, Giampaoli S, et al. Food Consumption Patterns in the 1960s in Seven Countries. *Am J Clin Nutr.* (1989) 49:889–94. doi: 10.1093/ajcn/49.5.889
74. Castro-Quezada I, Román-Viñas B, Serra-Majem L. The mediterranean diet and nutritional adequacy: a review. *Nutrients.* (2014) 6:231–48. doi: 10.3390/nu6010231
75. Romagnolo DF, Selmin OI. Mediterranean diet and prevention of chronic diseases. *Nutr Today.* (2017) 52:208–22. doi: 10.1097/NT.0000000000000228
76. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I, et al. Weight loss with a low-carbohydrate, mediterranean, or low-fat diet. *N Engl J Med.* (2008) 359:229–41. doi: 10.1056/NEJMoa0708681
77. Elhayany A, Lustman A, Abel R, Attal-Singer J, Vinker S. A low carbohydrate mediterranean diet improves cardiovascular risk factors and diabetes control among prospective randomized intervention study. *Diabetes Obes Metab.* (2010) 12:204–9. doi: 10.1111/j.1463-1326.2009.01151.x
78. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. *N Engl J Med.* (2018) 378:e34. doi: 10.1056/NEJMoa1800389
79. Couto E, Boffetta P, Lagiou P, Ferrari P, Buckland G, Overvad K, et al. Mediterranean dietary pattern and cancer risk in the EPIC cohort. *Br J Cancer.* (2011) 104:1493–9. doi: 10.1038/bjc.2011.106
80. Salas-Salvado J, Bullo M, Estruch R, Ros E, Covas M-I, Ibarrola-Jurado N, et al. Prevention of diabetes with mediterranean diets: a subgroup analysis of a randomized trial. *Ann Intern Med.* (2014) 160:1–10. doi: 10.7326/M13-1725
81. Estruch R, Martínez-gonzález MA, Corella D, Salas-salvadó J, Chiva-blanch G, Fiol M. Effect of a high-fat mediterranean diet on bodyweight and waist circumference : a prespecified secondary outcomes analysis of the PREDIMED randomised controlled trial. *Lancet Diabetes Endocrinol.* (2019) 7:e6–17. doi: 10.1016/S2213-8587(19)30074-9
82. Singh PN, Sabaté J, Fraser GE. Does low meat consumption increase life expectancy in humans? *Am J Clin Nutr.* (2003) 78:S526–32. doi: 10.1093/ajcn/78.3.526S
83. Sinha R, Cross AJ, Graubard BI, Leitzmann MF, Schatzkin A. Meat intake and mortality: a prospective study of over half a million people. *Arch Intern Med.* (2009) 169:562–71. doi: 10.1001/archinternmed.2009.6.Meat
84. Marsh K, Zeuschner C, Saunders A. Health implications of a vegetarian diet: a review. *Am J Lifestyle Med.* (2012) 6:250–67. doi: 10.1177/1559827611425762
85. Leitzmann C. Vegetarian nutrition: past, present, future. *Am J Clin Nutr.* (2014) 100:S496–502. doi: 10.3945/ajcn.113.071365.496S
86. Craig WJ. Health effects of vegan diets. *Am J Clin Nutr.* (2009) 89:S1627–33. doi: 10.3945/ajcn.2009.26736N
87. Tonstad S, Yan R, Butler T, Fraser GE. Type of vegetarian diet, body weight, and prevalence of type 2 diabetes. *Diabetes Care.* (2009) 32:791–6. doi: 10.2337/dc08-1886
88. Bedford JL, Barr SI. Diets and selected lifestyle practices of self-defined adult vegetarians from a population-based sample suggest they are more "health conscious". *Int J Behav Nutr Phys Act.* (2005) 2:4–14. doi: 10.1186/1479-5868-2-4
89. Roberts SB, Urban LE, Das SK. Effects of dietary factors on energy regulation: consideration of multiple- versus single-dietary-factor models. *Physiol Behav.* (2014) 134:15–9. doi: 10.1016/j.physbeh.2014.04.024

90. Rosell M, Appleby P, Spencer E, Key T. Weight gain over 5 years in 21966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. *Int J Obes.* (2006) 30:1389–96. doi: 10.1038/sj.ijo.0803305
91. Silvera SAN, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow W-H, et al. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer.* (2008) 123:852–60. doi: 10.1002/ijc.23544
92. Bosetti C, Negri E, Franceschi S, Pelucchi C, Talamini R, La Montelle M, et al. Diet and ovarian cancer risk: a case-control study in Italy. *Int J Cancer.* (2001) 93:911–5. doi: 10.1002/ijc.1422
93. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, et al. Meat, fish, and colorectal cancer risk: the European prospective investigation into cancer and nutrition. *J Natl Cancer Inst.* (2005) 97:906–16. doi: 10.1093/jnci/dji164
94. Chen CW, Lin YL, Lin TK, Lin CT, Chen BC, Lin CL. Total cardiovascular risk profile of taiwanese vegetarians. *Eur J Clin Nutr.* (2008) 62:138–44. doi: 10.1038/sj.ejcn.1602689
95. de Biase SG, Fernandes SFC, Gianini RJ, Duarte JLG. Vegetarian diet and cholesterol and triglycerides levels. *Arq Bras Cardiol.* (2007) 88:32–6. doi: 10.1590/S0066-782X2007000100006
96. Bradbury KE, Crowe FL, Appleby PN, Schmidt JA, Travis RC, Key TJ. Serum concentrations of cholesterol, apolipoprotein A-I and apolipoprotein B in a total of 1694 meat-eaters, fish-eaters, vegetarians and vegans. *Eur J Clin Nutr.* (2014) 68:178–83. doi: 10.1038/ejcn.2013.248
97. Cross AJ, Ferrucci LM, Risch A, Graubard BI, Ward MH, Park Y, et al. Large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. *Cancer Res.* (2010) 70:2406–14. doi: 10.1158/0008-5472.CAN-09-3929.A
98. Lagiou P, Talamini R, Samoli E, Lagiou A, Ahrens W, Pohlabeln H, et al. Diet and upper-aerodigestive tract cancer in Europe: the ARCAGE study. *Int J Cancer.* (2009) 124:2671–6. doi: 10.1002/ijc.24246
99. Butler LM, Wu AH, Wang R, Koh W-P, Yuan J-M, Yu MC. A vegetable-fruit-soy dietary pattern protects against breast cancer among postmenopausal Singapore Chinese women. *Am J Clin Nutr.* (2010) 91:1013–9. doi: 10.3945/ajcn.2009.28572
100. Craig, W.J., Mangels, A.R. Position of the American dietetic association: vegetarian diets. *J Am Diet Assoc.* (2009) 109:1266–82. doi: 10.1016/j.jada.2009.05.027
101. Gilsing, A.M.J., Crowe, F.L., Lloyd-Wright, Z., Sanders, T.A.B., Appleby, P.N., Allen, N.E., Key, T.J. Serum concentrations of vitamin B12 and folate in British male omnivores, vegetarians, and vegans: results from a cross-sectional analysis of the EPIC-oxford cohort study. *Eur J Clin Nutr.* (2011) 64:933–9. doi: 10.1038/ejcn.2010.142.Serum
102. Allen, L.H. How common is vitamin B-12 deficiency? *Am J Clin Nutr.* (2009) 89:S693–6. doi: 10.3945/ajcn.2008.26947A
103. Ho-Pham, L.T., Nguyen, P.L.T., Le, T.T.T., Doan, T.A.T., Tran, N.T., Le, T.A., Nguyen, T. V. Veganism, bone mineral density, and body composition: a study in buddhist nuns. *Osteoporos Int.* (2009) 20:2087–93. doi: 10.1007/s00198-009-0916-z
104. Dror, D.K., Allen, L.H. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. *Nutr Rev.* (2008) 66:250–5. doi: 10.1111/j.1753-4887.2008.00031.x
105. Freire, R. Scientific evidence of diets for weight loss: different macronutrient composition, intermittent fasting, and popular diets. *Nutrition.* (2020) 69:110549–59. doi: 10.1016/j.nut.2019.07.001
106. Halberg, N., Henriksen, M., Söderhamn, N., Stallknecht, B., Ploug, T., Schjerling, P., Dela, F. Effect of intermittent fasting and refeeding on insulin action in healthy men. *J Appl Physiol.* (2005) 99:2128–36. doi: 10.1152/japplphysiol.00683.2005
107. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr.* (2005) 81:69–73. doi: 10.1093/ajcn/81.1.69
108. Horne BD, Muhlestein JB, Lappe DL, May HT, Carlquist JF, Galenko O, et al. Randomized cross-over trial of short-term water-only fasting: metabolic and cardiovascular consequences. *Nutr Metab Cardiovasc Dis.* (2013) 23:1050–7. doi: 10.1016/j.numecd.2012.09.007
109. LeCheminant JD, Christenson E, Bailey BW, Tucker LA. Restricting night-time eating reduces daily energy intake in healthy young men: a short-term cross-over study. *Br J Cancer.* (2013) 110:2108–13. doi: 10.1017/S0007114513001359
110. Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers : a randomized trial in young overweight women. *Int J Obes.* (2011) 35:714–27. doi: 10.1038/ijo.2010.171
111. Hoddy KK, Gibbons C, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, et al. Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting. *Clin Nutr.* (2016) 35:1380–5. doi: 10.1016/j.clnu.2016.03.011
112. Cho Y, Hong N, Kim K, Cho SJ, Lee M, Lee Y, et al. The effectiveness of intermittent fasting to reduce body mass index and glucose metabolism: a systematic review and meta-analysis. *J Clin Med.* (2019) 8:1645–55. doi: 10.3390/jcm8101645
113. Headland M, Clifton PM, Carter S, Keogh JB. Weight-loss outcomes: a systematic review and meta-analysis of intermittent energy restriction trials lasting a minimum of 6 months. *Nutrients.* (2016) 8:354–65. doi: 10.3390/nu8060354
114. Salgin B, Marcovecchio ML, Humphreys SM, Hill N, Chassin LJ, Lunn DJ, et al. Effects of prolonged fasting and sustained lipolysis on insulin secretion and insulin sensitivity in normal subjects. *Am J Physiol Endocrinol Metab.* (2009) 296:E454–61. doi: 10.1152/ajpendo.90613.2008
115. Khalil MT. Impact of a detox diet paradigm in weight management. *Izzivi Prihodnosti.* (2017) 2:237–55.
116. Allen J, Montalto M, Lovejoy J, Weber W. Detoxification in naturopathic medicine: a survey. *J Altern Complement Med.* (2011) 17:1175–80. doi: 10.1089/acm.2010.0572
117. Cabot S. *The Liver Cleansing Diet: Love Your Liver and Live Longer*. Phoenix, AZ: SCB International (2014).
118. Woloshyn T. *The Complete Master Cleanse: A Step-by-Step Guide to Maximizing the Benefits of The Lemonade Diet*. Berkeley: Ulysses Press (2021).
119. Junger A. *Clean: The Revolutionary Program to Restore the Body's Natural Ability to Heal Itself*. New York, NY: HarperOne (2009).
120. Deluz R, Hester J, Beard H. *21 Pounds in 21 Days: The Martha's Vineyard Diet Detox*. New York, NY: Harper Collins (2009).
121. Cook MS. *Weekend Wonder Detox: Quick Cleanses to Strengthen Your Body and Enhance Your Beauty*. New York, NY: Da Capo Lifelong Books (2014).
122. Gittleman AL. *The New Fat Flush Plan*. New York, NY: McGraw Hill (2016).
123. Sakoutis Z, Huss E. *The 3-Day Cleanse: Your BluePrint for Fresh Juice, Real Food, and a Total Body Reset*. New York, NY: Grand Central Life & Style (2010).
124. Hubbard LR. *Clear Body Clear Mind: The Effective Purification Program*. Los Angeles: Bridge Publications (2002).
125. Genuis SJ, Birkholz D, Rodushkin I, Beesoon S. Blood, urine, and sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements. *Arch Environ Contam Toxicol.* (2011) 61:344–57. doi: 10.1007/s00244-010-9611-5
126. Wing RR, Phelan S. Long-term weight loss maintenance. *Am J Clin Nutr.* (2005) 82:S222–5. doi: 10.1093/ajcn/82.1.222s
127. Sainsbury A, Zhang L. Role of the arcuate nucleus of the hypothalamus in regulation of body weight during energy deficit. *Mol Cell Endocrinol.* (2010) 316:109–19. doi: 10.1016/j.mce.2009.09.025
128. Pankevich DE, Teegarden SL, Hedin AD, Jensen CL, Bale TL. Caloric restriction experience reprograms stress and orexigenic pathways and promotes binge eating. *J Neurosci.* (2010) 30:16399–407. doi: 10.1523/JNEUROSCI.1955-10.2010
129. Mazurak N, Gunther A, Grau FS, Muth ER, Pustovoyt M, Bischoff SC, et al. Effects of a 48-h fast on heart rate variability and cortisol levels in healthy female subjects. *Eur J Clin Nutr.* (2013) 67:401–6. doi: 10.1038/ejcn.2013.32
130. Tomiyama AJ, Mann T, Vinas D, Hunger JM, DeJager J, Taylor SE. Low calorie dieting increases cortisol. *Psychosom Med.* (2010) 72:357–64. doi: 10.1097/PSY.0b013e3181d9523c.Low
131. Torres SJ, Nowson CA. Relationship between stress, eating behavior, and obesity. *Nutrition.* (2007) 23:887–94. doi: 10.1016/j.nut.2007.08.008
132. Joint WHO/FAO/UNU Expert Consultation. *Protein and Amino Acid Requirements in Human Nutrition*. Geneva: World Health Organization (2007).

133. Food and Agriculture Organization [FAO]. *FAO Methodology for the Measurement of Food Deprivation: Updating the Minimum Dietary Energy Requirements*. (2008). Available online at: https://www.fao.org/fileadmin/templates/ess/documents/food_security_statistics/metadata/undernourishment_methodology.pdf (Accessed June 20, 2021).
134. Sanchez B, Casalots-Casado J, Quintana S, Arroyo A, Martin-Fumadoc C, Galtes I. Fatal manganese intoxication due to an error in the elaboration of epsom salts for a liver cleansing diet. *Forensic Sci Int*. (2012) 223:e1–14. doi: 10.1016/j.forsciint.2012.07.010

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REVIEW
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Dietary-Nutraceutical Properties of Oat Protein and Peptides

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Oats are considered the healthiest grain due to their high content of phytochemicals, dietary fibers, and protein. In recent years, oat protein and peptides have gained popularity as possible therapeutic or nutraceutical candidates. Generally, oat peptides with bioactive properties can be obtained by the enzymatic hydrolysis of proteins and are known to have a variety of regulatory functions. This review article focused on the nutraceutical worth of oat proteins and peptides and also describes the application of oat protein as a functional ingredient. Outcomes of this study indicated that oat protein and peptides present various therapeutical properties, including antidiabetic, antioxidant, antihypoxic, antihypertensive, antithrombotic, antifatigue, immunomodulatory, and hypocholestrolaemic. However, most of the conducted studies are limited to *in vitro* conditions and less data is available on assessing the effectiveness of the oat peptides *in vivo*. Future efforts should be directed at performing systematic animal studies; in addition, clinical trials also need to be conducted to fully support the development of functional food products, nutraceutical, and therapeutical applications.

Keywords: oat protein, bioactive peptides, antioxidant, antidiabetic, antihypertensive, immunomodulatory, antifatigue, antihypoxic

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INTRODUCTION

Oats are one of the most nutritious grains and are the 5th most consumed crop with an annual production of 23 million tons globally (1, 2). Generally, oat grains can be classified as naked oats (*Avena nuda* L.) and hulled oats (*Avena stiva* L.). Oats contain a relatively high content of protein, and it also comprises a considerable amount of lipids and other bioactive compounds, such as beta-glucans, phenolics (trace–150 mg/kg), avenanthramides (26–150 mg/kg), and flavonoids 1.77 mmol/g (3, 4). Depending on different varieties, oat grain comprises lipids 5–10%, crude protein 12–20%, crude fiber 3–14%, and carbohydrates 69–76% (5, 6). The protein content of oat grain is higher as compared to other cereals, including rice 7–10%, wheat 11–15%, and millets 7–11%, while lower than legumes, such as pea 23–31% and soy 36–40%. The protein fractions and their molecular weights are also significantly different among grains as given in **Table 1** (6–10).

Globulin is the major storage protein in oats. Mainly, oat protein is composed of four fractions; globulin 70–80%, albumins 1–12%, prolamins 4–15%, and glutenins $\leq$ 10% (18). The salt soluble globulin fraction is divided into 3 subunits, 12S, 7S, and 3S. The molecular weight of globulin ranges from 54 to 60 kDa, which is approximate to the 11S globulin (glycinin) structure of soy protein (12, 13); besides this, 12S has acidic A and basic B polypeptides subunits with a molecular weight of

32 and 22 kDa, respectively (19). Other subunits of globulins, 7S has polypeptides of molecular weight of 55–65 kDa, and 3S contains two polypeptides of 15 and 21 kDa (17, 18). Water-soluble albumin plays a role as an enzyme in the protein and defensive system of plants, and its molecular weight ranges from 19 to 21 kDa (20). Prolamin (avenins), the alcohol-soluble protein fraction with a molecular weight of 20–40 kDa, has structural similarities with Sulfur rich sub-units of wheat (α and γ gliadins), γ-secalins of rye, and B-hordeins of barley proteins fractions (12, 19). This protein fraction is limiting in proline and glutamine amino acids but has the same function as the storage protein fraction (gluten) of wheat (21). The minor fraction (Glutelins) comprises polypeptides having a molecular weight of 10–90 kDa (20).

Oat protein contains a comparatively higher amount of essential amino acids, especially (lysine, valine, isoleucine, threonine, histidine, and methionine) than other grains. Amino acids composition of oat protein significantly differs even among its fractions, globulins carry the highest amount of essential amino acids, such as lysine, valine, phenylalanine, and histidine, as well as non-essential amino acids, including arginine and glutamic, as compared to all three other fractions (20). In addition, oat amino acids composition meets the Food and Agriculture Organization's recommended nutritional needs of an adult except for methionine, which is still the limiting amino acid in oat protein (22). Furthermore, the consumer's acceptability score of oat protein is also higher than any other plant-based pea, lupin, and soy proteins (20, 23, 24).

Plant protein has received much attention in recent years, as consumers are pursuing more plant-based diets in hopes of improving health and preserving the environment. Livestock puts much higher pressure on global warming, resource, and land use than plant-based food (25). Animal-based foods, including dairy, eggs, meat, and aquaculture, use 83% of the world's cultivated land and contribute up to 58% of food emissions, while providing only 37% of the total proteins (26). The higher environmental impact of animal products is due to the high feed consumption per kilogram of food produced, in addition, a large part of greenhouse gas emissions comes from intestinal fermentation and fecal management in ruminants (26, 27). There is a need to reduce greenhouse gas emissions by minimizing the animal-based proteins, and substituting even only a part of animal protein intake with plant-based proteins would benefit both human health and the environment (28). Numerous studies have shown that plant products have less impact on climate and eutrophication and lower land use as compared to animal-based products. For example, beans and peas have the lowest land use per kg of protein as compared to milk, eggs, and poultry (29–31). Specifically, the carbon footprint of oat protein concentrate (OPC) is more than 50% lower when compared with dairy proteins (32). OPC-enriched food products could reduce 13% of greenhouse gas emissions and 26% of land use when substituted with animal-based proteins (33); similarly, oat drinks emitted 16–41% lesser greenhouse gas as compared to cow milk (34). The use of plant proteins has considerable potential in mitigating climate change and reducing land use. From this perspective, the above-mentioned studies reveal the potential that the oat protein made it possible to substitute some animal proteins with plant proteins.

Being of high protein content, sustainability, as well as good nutritional profile, oats are considered the promising cereals for extracting plant-based proteins and isolating bioactive peptides (35). Plant bioactive peptides, in general, are small fragments of proteins, composed of 2–20 amino acids and having less than 3 kDa molecular weight (36, 37). These peptides naturally exist or are derived from precursor proteins through gestational simulation, microbial fermentation, or enzymatic hydrolysis (38). It has been found that bioactive peptides have a simpler structure, higher stability, and more remarkable physiological activities and functions compared to protein (39). In this way, oat protein-derived bioactive peptides have been identified to exert distinct health improving properties, such as antidiabetic, immunomodulatory, antifatigue, antithrombosis, antihypoxic, antihypertensive, hypocholestrolaemic, and antioxidant effects (**Figure 1**). In this article, we mainly focus on the oat proteins in detail for their application as a functional ingredient and potential nutraceutical activities (**Table 2**).

APPLICATION OF OAT PROTEIN AS A FUNCTIONAL INGREDIENT

Interest in protein-rich diets is increasingly favored by their health-promoting effects, with a special focus on sources. Plant-based protein is replacing animal protein as it is considered better for human consumption, as well as safe for the planet (40). The market request for developing plant protein-based products is continuously increasing because of high concern toward health, animal welfare, and environmental protection. This searching desire for alternative non-animal protein sources opens the way to the valorization of non-fully exploited plants and industrial by-products (33). With the increase in demand for nutrition products, oat protein-infused beverages, bakery items, supplements, and others are making their way into the market, as it contains high-quality protein and is also suitable for flavoring. Numerous studies have been conducted to review the possibility of using oat for the development of nutraceuticals. The lipid-lowering and antioxidant properties of oat protein make it a potential ingredient for nutraceuticals (41). Oat protein (OP) gels have been used for the preparation of prebiotic loaded nutraceuticals which can prevent the deterioration of prebiotics in gastric conditions. OP gels have been shown to resist pepsin digestion and have the capacity to release bioactive compounds during gastrointestinal simulation conditions (19). Due to growing interest in plant protein ingredients, oat has gained much attention for its unique amino acid composition, protein quality, and protein content. The global oat protein market reached US$ 48 million in 2018 and is expected to grow up to US$ 63 million by the end of 2025 with a compound annual growth rate of 4.1% during 2019–2025 (42). Regarding usage and composition, oat protein increased from 884t in 2012 to 1398 t in 2017 globally, with a compound annual growth rate of more than 9.6% (43).

TABLE 1 | Protein content and molecular weight distribution of oat and other grains.

Grains	Total protein	Globulins		Albumins		Prolamins		Glutenins	
		%	MW	%	MW	%	MW	%	MW
Oat	12–20%	70–80	54–60 kDa	1–12	19–21 kDa	4–15	20–40 kDa	≤10	10–90 kDa
Wheat	11–15%	*A/G 20–25	98–100 kDa	*A/G 20–25	15 kDa	30–40	35 kDa	45	10 million kDa
Rice	7–10%	7–17	23–105 kDa	5–10	10–200 kDa	3–6	10–32 kDa	75–81	51–57 kDa
Millet	7–11%	*A/G 11–17	13–32 kDa	*A/G 11–17	13–32 kDa	*6.8–9.3 P like; 7.5–11.6	12–35 kDa	*39–54 G like; 5.9–9	10.5–56 kDa
Pea	23–31%	2.47	10–43 kDa	7.01	11–21 kDa	1.52	–	87.47	12–66 kDa

**A/G, Albumin and Globulin combine; P like, Prolamine like fraction; G like, Glutenins like fraction (11, 14–17).*

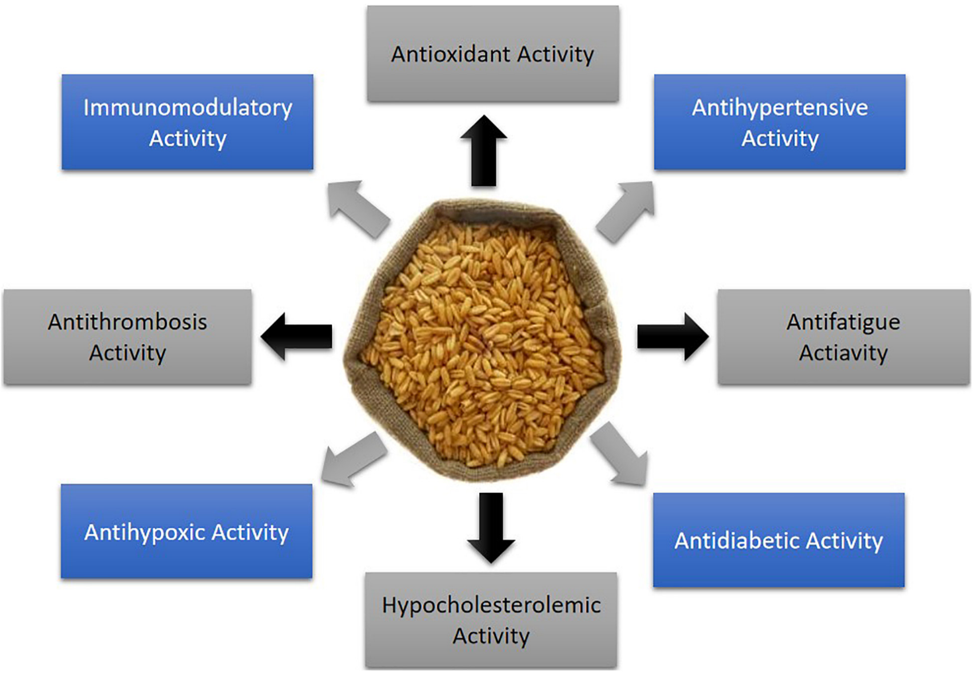

FIGURE 1 | Health protecting properties of oat derived bioactive peptides.

In terms of using oat protein in food applications, oat protein has better sensory properties than legume and oilseed proteins (44). Oat protein concentrates and isolates can be easily incorporated into pasta and bakery products to improve their protein content. Although well suited to bakery products, the applicability of oat protein is still limited in semisolid and liquid foods because of its low solubility in natural and mildly acidic conditions (45). Oat protein is also a good replacer of skim milk powder because of its ability to produce better quality yogurt in terms of mouthfeel and syneresis. OPC due to the excellent techno-functional properties of the contained oat starch is an appropriate ingredient for the implementation of the nutritional value of oat protein, giving a healthier and more natural shape to the yogurt (46). Given the increased demand for a plant protein source, meat analogs produced from plant proteins have gained traction (47). The meat analogs' market has long been dominated by pea, wheat gluten, and soy proteins (28). However, due to the presence of common allergens in pea and soy protein, the oat protein has the potential to play a significant role in the meat alternative market as a new protein ingredient (48). Besides, using oat protein in meat alternative analog or yogurt, oat milk is also included in the list of common oat products that dominate the market. The oat milk is a promising substitute for dairy products because it can be used in combination with probiotics to prepare fermented products like yogurt. Due to consumer awareness about plant-based milk and increased interest in flexitarian, vegan, and vegetarian diets, the international oat milk market reached US$ 4 billion in 2020 and is expected to grow with a compound annual growth rate of 9.8% by 2027 (49).

The presence of many substitutes like soya, whey, and others, which contain protein in heavy quantities and are demanded by the nutraceutical companies, lead to increased competition for oat protein products. In terms of food application, few oat protein products are available in the market given in **Table 3**, which urges food developers to build tailored strategies and food portfolios of these ingredients. As a functional food ingredient, oat protein application is still in the early stages, and food companies are investing in the production of high-quality oat protein products (44).

NUTRACEUTICAL PROPERTIES OF OAT PROTEIN-DERIVED PEPTIDES

Antioxidant Activity

Oats are known to contribute to a significant supply of antioxidants in the form of phytic acid, phenolic compounds, vitamin E, and avnanthramides to counter oxidative stress (50). The antioxidant defense system works to balance the reactive oxygen species by superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), vitamins, minerals, and co-factors to prevent cellular oxidative stress. These antioxidant compounds demonstrated radical scavenging activities by participating in a single electron transfer reaction (51). Oat proteins have also been considered a good source of antioxidant capacity, which have the potential to strengthen the treatment of oxidation-linked disorders, delay the oxidation process in foods and improve quality of life (52). Oat protein isolates and hydrolysates exhibited excellent antioxidant activities when assessed for 2,2 Azino-Bis-Ethylbenzoline-6-Sulfonic acid (ABTS), (HO**.**), (O_2**.**), and $Fe(2^+)$ chelating assays (53). Bioactive peptides (IRIPIL, FLKPMT, FNDILRRGQLL, LIGRPIIY, and NSKNFPTL) isolated from globulin fraction had the strongest 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (IC_{50} = 4.11 ± 0.07 mg/mL) and hydroxyl (IC_{50} = 1.83 ± 0.03 mg/mL) radical scavenging activity (54). In another study, oat peptides (GLVYIL, YHNAP, and GQTV) showed cytoprotective and peroxyl radical scavenging potential with considerable oxygen radical absorbance capacity (ORAC) value 0.67 ± 0.02, 0.61 ± 0.04, and 0.52 ± 0.01 Trolox equivalent (TE)/μM, respectively. The cytoprotective activity of peptides GLVYIL, YHNAP, and GQTV was correlated with the overall hydrophobicity of peptides, but not with their ORAC values (55).

Oat hydrolysates exhibited a cytoprotective effect by protecting $HepG_2$ cells from AAPH-induced oxidative stress by significantly magnifying the activities of antioxidant enzymes, including GPx, CAT, and SOD, and inhibiting reactive oxygen species (ROS) levels (56). Furthermore, lipid oxidation and linolenic acid peroxidation were significantly inhibited up to 52, 35, and 16% with NINAHSVVY, YFDEQNEQFR, and SPFWNINAH peptides, respectively (57). The presence of tyrosine on (N or C-terminal) and histidine in a sequence of peptides improved their activity, as both these amino acids have good electron or proton donating ability. Hydrophobicity is also an important factor to interact with lipids (57). A recent development to alleviate anemia and oxidative stress has been made by using oat antioxidant peptide as a carrier to synthesize a novel oat peptide-ferrous ($OP\text{-}Fe^{+2}$) chelate. $OP\text{-}Fe^{+2}$ has shown to be helpful to alleviate anemia and also improved the activities of antioxidant enzymes, including GSH and SOD, while limiting the malondialdehyde (MDA) content in the liver of iron-deficient anemic rats model (58). In addition, oat peptides effectively recovered the H_2O_2 induced apoptosis and oxidative stress in human dermal fibroblast by regulating cellular antioxidant enzymes (59). Collectively, these reports demonstrated that oat-derived peptides can reduce oxidative stress and related disorders and may contribute to the development of nutraceutical or functional foods.

Antidiabetic Activity

Oat peptides have the potential to inhibit dipeptidyl peptidase-4 (DPP4) and α-amylase and enhance the release of glucagon-like peptide-1 (GLP-1), as shown in **Figure 2**. Numerous dietary bioactive peptides work as antidiabetic agents by regulating insulin resistance and energy metabolism (60). Oat globulin peptides exhibited DPP4 inhibitory activity *in vitro*, with half-maximal inhibitory concentration (IC_{50}) values of DPP4 at 100.4 μg/mL for peptide LQAFEPLR and 2.04 mg/mL for oat tryptic hydrolysates (61). Oat peptides showed improved DPP4 inhibition than fish peptides (42.5% at concentration of 5 mg/ml) and milk peptides IC_{50} = 0.60–2.14 mg/mL (55, 57, 62). Oat peptides inhibited the activity of DPP4 through binding to its active site with low binding energy and also downregulated the protein expression in Caco-2 cells. In addition, oat globulin peptides suppressed the α-glucosidase with IC_{50} of 35.67 μg/mL for LQAFEPLR and 113.8 μg/mL for hydrolysates (61). Walters et al. (63) have evaluated the antidiabetic potential of oat hydrolysates in NCI-H716 cells. The results indicate a significant inhibition of DPP4 (30.6–53.6%) and α-amylase (18–32%), and also improved insulin secretion, as well as glucose digestion. The inhibition mechanism has not been clarified, and a previous review (64) has shown that the inhibition of α-amylase by food-derived peptides usually occur through competitive binding between polysaccharide and peptides mainly through their aromatic amino acids. Another factor GLP-1 contributes to the stimulation of insulin, inhibition of glucagon by acting as an incretin hormone, and limits the postprandial glucose level. The secretion of glucagon-like peptide (GLP-1) was also improved in NCI-H716 cells, which ranges from 20.85 to 39.25 pM (picomole) (63). It has been shown that food-derived peptides affect gene expression, bile acid activation, and calcium receptors (65). Hence, peptidomics profile of hydrolysates is necessary to confirm their potential contribution to activity.

In another study, the peptides YFDEQNEQFR, NINAHSVVY, and RALPIDVL potentially inhibited α-amylase *in vitro*, with IC_{50} values of 37.5, 67.3, and 72.8 μM, respectively. The most active α-amylase inhibitory peptide was (YFDEQNEQFR) with acidic properties and containing tyrosine (Y) and arginine (R) amino acids, which may have contributed to the highest activity (57). Furthermore, oat peptides have been shown to effectively improve the symptoms of polydipsia, weight loss, and polyphagia, which can control the blood glucose level by improving insulin sensitivity and promoting glycogen synthesis (66). Oat grains and their bioactive compounds have been extensively studied for antidiabetic significance in human subjects. Regular oats intake for 23 weeks can improve fasting blood glucose level and insulin concentration by up to 20%, meanwhile, beta-glucans supplementation improves the blood glucose, insulin GLP-1, HbA1c, and appetite-regulating hormones in type 2 diabetic individuals (67, 68). However, work on the antidiabetic

TABLE 2 | Oat protein derived hydrolysates/peptides and their bioactivity.

Protein fraction	Protease	Hydrolysates/peptides	Bio activity	References
Total protein	Alcalase Flavourzyme Papain, protamex	Hydrolysates	Exhibited the hydroxyl, peroxyl, ABTS radical scavenging, and Fe 2^{+} chelating activities *in vitro*	(53)
Globulin protein	Alcalase	IRIPIL, FLKPMT, NSKNFPTL, LIGRPIIY, FNDILRRGQLL	Exhibited DPPH and hydroxyl radical scavenging activity *in vitro*	(54)
Total protein	Peptides Synthesized by GenScript	FNDRLRQGQLL, GLVYIL, GQTV, GQTVFNDRLRQGQLL, YHNAP, YHNAPGLVYIL, DVNNNANQLEPR	Displayed the Peroxyl radical scavenging (ORAC) and cytoprotective capacity in stressed HepG2 hepatic cells	(55)
Total protein	Flavourzyme Papain Alcalase	Hydrolysates	Showed Peroxyl (ROO.), superoxide (O.), and hydroxyl (HO.) radical scavenging activity *in vitro*	(63)
Total protein	Alcalase, Papain, Flavourzyme, Protamex	Hydrolysates, pretreated with cellulose degrading enzymes	Improved the activities of Antioxidant enzymes, including CAT, SOD, and GPx, in stressed induced hepatic cells	(56)
Oat bran	–	Oat peptide-ferrous (OP-Fe^{+2}) chelate	Alleviated the oxidative by increasing the activity of SOD and GSH and down-regulating MDA content in rats	(58)
Oat bran extract		Oat peptides	Reversed the H_2O_2 induced decrease of superoxide Dismutase and inhibited malondialdehyde in Human dermal fibroblast	(59)
Total protein	Papain	YFDEQNEQFR, GQLLIVPQ, SPFWNINAH, NINAHSVVY, RALPIDVL	Inhibited the lipid oxidation and linoleic acid peroxidation also inhibited the α-amylase *in vitro*	(57)
Globulin protein	Trypsin	Hydrolysates And LQAFEPLR, EFLLAGNNK	Competitively suppressed the DPP4 and alfa-glucosidase downregulated the protein expression of DPP4, while elevated the protein expression of α-glucosidase, GLUT2 and GLUT 4 in Caco-2 cell lines	(61)
Total protein	Flavourzyme Papain,Alcalase	Hydrolysates	Inhibited the dipeptidyl peptidase-4 and α-amylase. Improved the secretion of glucagon like peptide-1 in NCI-H716 cell lines	(63)
Total protein	Chemically synthesized	Protein, FFG, IFFFL, PFL, WCY, YPIL, CPA, FLLA, and FEPL	Inhibited the secretion of Angiotensin-1 converting enzyme and Renin *in vitro*	(70)
Globulin	Alcalase, Flavourzyme, Pepsin, Trypsin	SSYYPFK selected based on *in silico* analysis	Inhibited the activity of Angiotensin-1 converting enzyme and Renin and ET-1 *in vitro*	(72)
Total protein	Multiple proteases	Mixture of Oligo-peptides	Improved innate and adaptive immunity *via* regulating Cytokine's secretion, antibody production and T cells stimulation in rat's model	(75)
Total protein	–	Oligopeptides	Improved the hypoxia by regulating LDH, MDA, HB, HCT, RBC, VEGF, and mRNA expression in rats' model	(81)
Total protein	–	Oatmeal, protein isolates	Effectively improved the fatigue by increasing liver glycogen, SOD, LDH, and reducing the BUN and MDA in rats' model	(88)
Total protein	–	Oat protein isolates	Alleviated the exercise induced fatigue by reducing plasma myoglobin, IL-6, creatinine kinase, and C reactive protein content. Also inhibited the limb edema following damaging exercise and lessened the adverse effects on muscle strength in human clinical trial	(95)
Total and globulin protein	Pepsin Pencreatin and trypsin	Hydrolysates and peptides	Inhibited the Arachidonic acid induced platelet aggregation by acting on COX1-TXA_2 synthase pathway to produce TXA_2 *in vitro*	(100)
Total Protein	–	Protein isolates	Increased the excretion of total cholesterol and bile acids, consequently decreased in plasma level of low-density lipoprotein, liver total cholesterol and activity of liver 7α-hydroxylase (CYP7A1) increased in animal model	(103)

potential of oat peptides is still limited to *in vitro* studies; thus, systematic *in vivo* research is needed to fully understand the molecular mechanism behind the antidiabetic properties of oat bioactive peptides.

Antihypertensive Activity

Oat's bioactive peptides have been shown to exert antihypertensive activity by targeting the renin–angiotensin system (RAS) (**Figure 3**). Oat protein isolates exhibited significant renin inhibitory activity, ranging from 40.50 to 70.5%, while the synthesized peptides showed comparatively lower inhibition than protein isolates. The attributes of renin inhibitory peptides are not clearly defined like ACE inhibitory peptides (69). Among the peptides, only IFFFL took part in renin inhibition due to the presence of bulky amino acids on the N-terminal, while the higher renin inhibitory activity of protein isolates could also be due to the presence of other bioactive compounds in protein, such as phenolic compounds (70). Oat protein isolates

TABLE 3 | Oat protein products available in the market.

Product name	Product type	Characteristics	Links
PrOatein Havreprotein	Oat proteins concentrate powder	Have mild taste of oat Suitable for cooking, baking and can be mixed with liquid or in smoothie. Contain no additive	https://www.apotea.se/proatein-havreprotein-450-g
Ideal oats® Oat protein	Oat protein concentrate	Naturally contain all essential amino acids, including desirable BCAA'S, can help to boost up metabolism, muscle growth, tissue repair and may lower the cholesterol	https://www.idealoats.com/pages/plant-based-oat-protein-powder
Critical oats	Advanced protein porridge	Breakfast snack food supplement contains protein isolates. It can help to increase energy, improves digestion and nutrients absorption	https://appliednutrition.uk/products/critical-oats
Protein oat	Protein oat milk	Protein rich, creamy oat milk with 8 g of plant-based protein, calcium and vitamin D. Can be directly use as drink, pour over cereals, or add to smoothies.	https: //www.califiafarms.com/products/original-protein-oat-milk-48oz
Muscle feast oats + isolates	Oats and whey protein powder	Natural, hormones and gluten free Oat and whey protein powder provide 31 g protein per serving. Protein isolates can be mixed with water or other beverages. Suitable for muscle growth and tissue repair.	https://www.musclefeast.com/products/oats-and-whey
Protein oats Oatmeal drink mix	Oat drink	Oatmeal drink mix can used in form of shake after workout to keep gut healthy, improve immunity and to faster muscle recovery	https://www.navafresh.com/products/borito-whey-protein-oats-belgian-chocolate
Oats and whey	Powder product	Oats and whey powder can be used with milk or water. It contains 35% of protein, contributes to the growth of muscle mass and also limits the catabolic process on muscles in muscle cells. Ideal to use before or after workout meal	https://ostrovit.com/en/products/ostrovit-oats-whey-1000-g-24313.html
Optimum nutrition oats and whey	Drinkable protein shake	Drinkable shake, easy to prepare and provide 24 g of protein per serving. Made up of whole oat flour and whey, free from artificial flavor, color and sweeteners. Contains fundamental elements for an athlete's diet.	https://www.aasportsnutrition.com/product/optimum-nutrition-oats-and-whey/

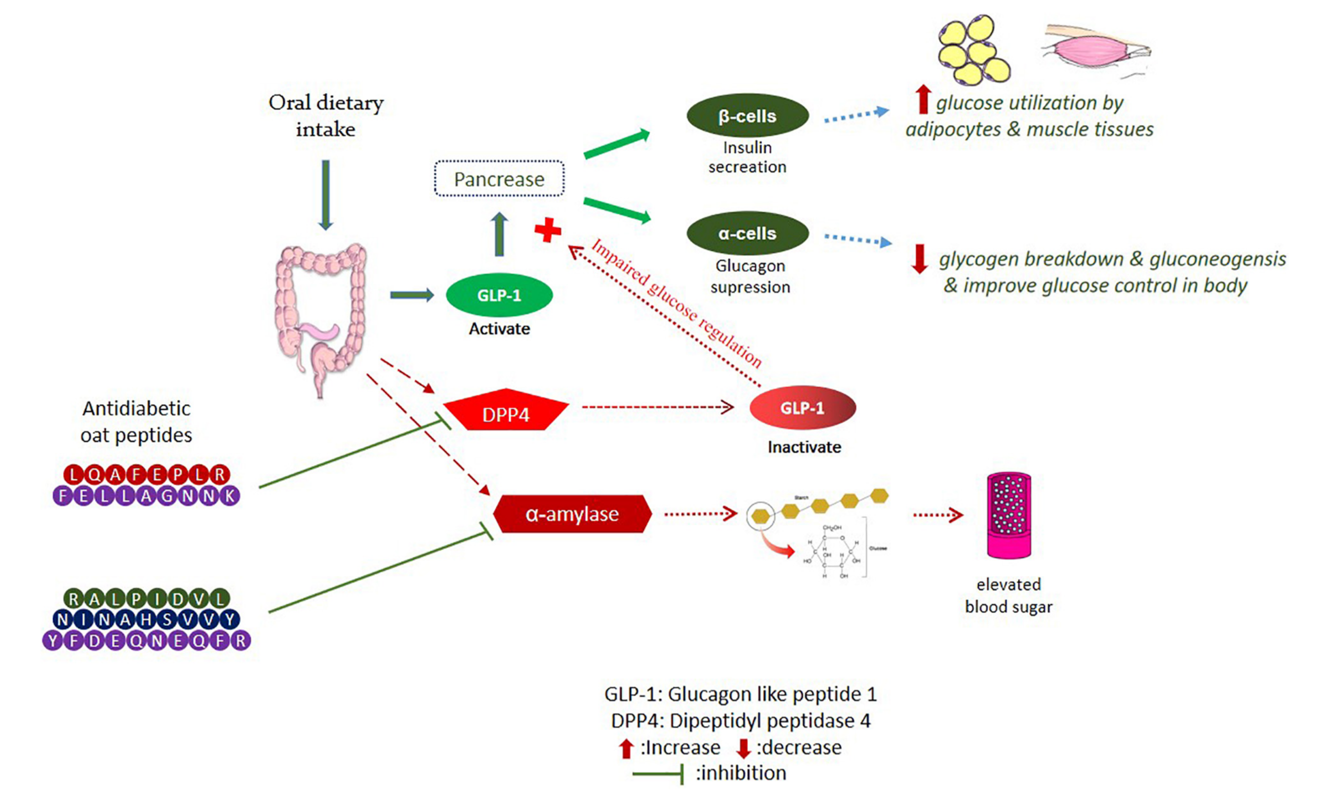

FIGURE 2 | Inhibition of DPP4 and α-amylase pathways by oat bioactive peptides.

and peptides significantly inhibited the ACE, and protein isolates were found to inhibit ACE between 86.6 and 96.5%. The highest ACE inhibition value of protein is comparable with the positive control (captopril), which was found to inhibit the ACE by 97.7%. The synthesized peptides WCY, FLLA, and WWK were recorded for their highest ACE inhibition by 97.8, 97, and 95.3%, respectively. Peptide FEPL showed the lowest ACE inhibition value of 48.9% (70), and the lower ACE inhibition capacity of FEPL was due to the presence of proline, which has been suggested to reduce the binding affinity of the peptide with angiotensin-converting enzyme (ACE) (71).

Oat globulin peptide (SSYYPFK) was found to lower the systolic and diastolic blood pressure in a hypertensive rat model and exhibited high ACE inhibitory activity (IC_{50}: 91.82μM). In addition, peptide SSYYPFK inhibited the renin by 28.6–45.59% and suppressed the production of intracellular endotheline-1 (ET-1) by 13.19–27.88% (72). Overexpression of ET-1 is an endogenous mediator of cardiovascular problems, such as hypertension or atherosclerosis. ET-1 and nitric oxide are important systems involved in the regulation of blood pressure, and peptide SSYYPFK may play an antihypertensive role by affecting these systems (73). Some clinical studies have been conducted on the antihypertensive effects of oatmeal, beta-glucan, dietary fibers, avenanthramides (AVA), and their fortified products. Most of these human interventional studies assessed the advantageous effects of oats and its derived active compounds in hypertensive and normotensive subjects by regulating systolic or diastolic blood pressure (74). Above mentioned research outcomes have confirmed that oat protein-derived peptides could effectively inhibit ACE and renin by targeting respective pathways and may be used as a functional ingredient for the prevention of hypertension and related disorders after interventional trials.

Immunomodulatory Effects

Oat bioactive peptides exhibited immunomodulatory activity by improving innate and adaptive immunity. Oat oligopeptides (OOPs) attributed to the significant enhancement in interleukin IL, serum interferon (IFN)- γ, tumor necrosis factor (TNF)-α, T and Th cells percentage, immunoglobulin IgA, IgG production, as well as granulocyte macrophages colony-stimulating factors (GM-CSF) secretions (75). It also found that after OOP's treatment, the percentage of $CD3^+$ and $CD4^+$ were enhanced, indicating the improvement in T cells quantity, which results in cytokines secretions and mediated cellular immune response (75). Specifically, CD4-T cells consists of T helper-1 (Th1) cells, which induce a cell-mediated immune response by producing IL-2, TNF-α, INF- γ, T-helper 2 (Th2), and GM-CSF to induce humorous responses by secreting IL-4, IL-5, and IL-10 cytokines (76). A previous review has shown that oat gluten protein and peptides can significantly enhance the ability of ConA-induced splenic lymphocyte transformation and delayed-type allergy. Meanwhile, a combination of oat peptides with American ginseng peptides has been shown to significantly improved the mice's immunity (77). Hence, oat bioactive peptides are a potential source to improve immunity; however, the exact mechanism through which food-derived bioactive peptides regulate the immune system remains unclear.

Antihypoxic Effects

Bioactive peptides from plant and animal sources have received much attention for their potential role in preventing hypoxia and other metabolic disorders (78–80). Oat oligopeptides (OOPs) can effectively ameliorate Hb, RBC, and Hct levels, which improves the oxygen-carrying capacity, as well as oxygen utilization rate of blood (81). The oxygen-carrying capacity of blood is directly reflected by the number of red blood cells and (HCT) indicating the volume ratio of RBCs to whole blood. Oxygen is attached to an iron atom and transported to the whole blood, and hemoglobin (Hb) combines with oxygen to transfer it from higher content areas to lower one's, where it is needed (74, 77).

Oat oligopeptides also suppressed lactate and increased the activity of LDH, consequently upsurging the ability of the brain against lactic acidosis. The brain is more susceptible to oxidation because of its lipid membrane and weak antioxidant system. In the case of hypoxia, mitochondrial oxidase cannot completely reduce oxygen to water, resulting in the accumulation of reduced equivalent in the respiratory chain, which ultimately produces ROS. These factors trigger the lipid peroxidation in the brain and consequently, alkanes, epoxy fatty acids, alkanals, alkenals, and aldehyde, such as MDA, are produced (82–85). OOPs have been shown to decrease the MDA content of the brain, which minimizes lipid peroxidation and promotes angiogenesis, ultimately improving the hypoxic response (81).

Antifatigue Activity

Oat bioactive peptides can alleviate the exercise-induced fatigue by improving muscle strength (86). Lactic acid accumulation and acidosis are widely considered to cause an increase in hydrogen ion concentration, which leads to a decreased action potential, inhibited sarcoplasmic reticulum uptake, and the release of calcium ions (87). Oat peptides effectively upregulated lactate dehydrogenase and suppressed the production of lactic acid. In addition, oat peptides downregulated the blood urea nitrogen and increased the level of glycogen in the liver and muscles (86). In another study, oat protein isolates have shown to significantly improve the physiological condition of mice by increasing the level of liver glycogen up to (19.64%), enhancing the activities of superoxide dismutase SOD by 20.27% in blood and 81.32% in muscles, and lactic dehydrogenase LDH (13.58%), and decreasing the level of malondialdehyde MDA by 3.45% in blood and 53.12% in muscles, and blood urea nitrogen BUN by 18.25% (88). Normally, hepatic glycogen plays an important role to complement blood glucose consumption and maintain its level in the physiological range. In the case of intensive exercise, glycogen depletion severely limits energy supply and maximal power output. Consequently, fatigue may happen, when most of the glycogen is consumed from the liver (89, 90). Urea is formed as the end product of protein metabolism in the liver, and blood urea nitrogen (BUN) is another sensitive parameter of fatigue, which dramatically increases after intensive exercise (91–93). Furthermore, decreased level of lactate dehydrogenase (LDH) is a biomarker of muscle damage. It is an enzyme of the glycolytic pathway and is dependent on NAD^+ for the interconversion of pyruvate and lactate (94).

A clinical study confirmed that the supplementation of oat protein for 14 days prior and 4 post-exercise days alleviated the exercise-induced fatigue by preventing physical discomfort and reducing the elevation of plasma biomarkers, including creatine kinase, IL-6, C-reactive protein, and myoglobin content. In addition, oat protein prevented the decline of joint range of motion, jump performance, and muscle strength, and enhanced the post-exercise recovery of damaged muscles in healthy adults (95). These scientific studies depicted that oat protein can effectively alleviate exercise-induced fatigue.

Antithrombotic Activity

The arachidonic acid (AA) pathway plays an important role in the platelet's aggregation, AA directly acts on the COX1–TXA_2 synthase pathway to produce TXA_2. COX1 is a key enzyme in this pathway, associated with the metabolism of AA to induce TXA_2 formation (96, 97). Finally, TXA_2 causes changes in the shape of the platelet and activates fibrinogen receptors, consequently leading to platelet aggregation (98, 99). Hydrolysates produced from oats, barley, and buckwheat have been reported to have a crucial role in inhibiting platelet aggregation. Among all, oat hydrolysates showed high antiplatelet activity with an IC_{50} value of 0.282 mg/mL (100). Furthermore, oat globulin (small, mid, and large sized) fractions significantly inhibited the AA-induced platelet aggregation by (67.69, 17.98, and 14.33%) and increased the inhibitory rate to 73.11, 75.37, and 69.23%, respectively. These values are very close to the inhibitory rate of ASA (88.3%), which is a medical standard. The small-sized oat fraction showed more activity as compared to mid and large-size fractions (100). These results show that the inhibition of platelet aggregation by oat-derived bioactive peptides/hydrolysates could affectively act on COX1-TXA_2 synthase pathway and may be employed to manage thrombosis and related chronic disorders.

Hypocholesterolemic Effects

The cholesterol-lowering activity of oats is often attributed to its ability to reduce cholesterol absorption in the intestine or inhibit the enterohepatic circulation of bile acids by increasing the carriage of cholesterol and bile acid in the colon to facilitate their excretions through the feces. Oat contains numerous bioactive compounds to regulate cholesterol metabolism in animals (101). Hypocholestrolaemic activity of oat is strongly dependent upon their protein composition. A related study depicted a significant reduction in liver and plasma cholesterol levels of rats fed with hypercholestrolaemic diet by increasing fecal bile acids secretions (102). Oat protein significantly reduced the plasma low-density lipoprotein (LDL-C) and total cholesterol level in the liver by increasing the excretion of bile acids and regulating the liver CYP7A1 activity in an animal model (103). Regulation of specific genes and enzymes, such as LDLR, HMG CoA reductase, HYP3A4, and CYP7A1, is mainly involved in cholesterol homeostasis by converting cholesterol to bile acids or transporting it to hepatic cells and in bile acids metabolism (104).

A clinical study exhibited a significant reduction in triglycerides and total cholesterol of 268 hypercholesterolemic adults after consuming whole oat gain and β-glucan fortified products (105). Oat bran containing β-glucan was recorded for its remarkable effects in hyperchlesterolemic individuals and lowered the cholesterol by 23% without affecting the level of high-density lipoprotein HDL in the blood (106). Similarly, another clinical study claimed that the consumption of 6 g oat β-glucan for eight consecutive weeks effectively reduced the plasma LDL and total cholesterol (from 167.9 to 120.9 mg/dL and 231.8 to 194.2 mg/dL, respectively) and increased the plasma HDL from 39.4 to 49.5 mg/dL in overweight individuals with mild hypercholesterolemia (107). However, limited studies have been found on the hypocholestrolemic effects of oat protein and peptides.

PROCESSING AND PURIFICATION OF BIOACTIVE PEPTIDES

Processing of Bioactive Peptides

Bioactive peptides are defined as specific fragments of proteins that have positive effects on body functions and improve human health. The composition and sequence of amino acids determine the activity of peptides when they are released from a precursor protein, where they are encrypted (108). Generally, bioactive peptides are produced by microbial fermentation or by the enzymatic hydrolysis of protein. Several microorganisms, including *Streptococcus salivarius* ssp. *thermophilus, Lactobacillus helveticus, Lactococcus lactis* ssp. *diacetylactis, Lactobacillus delbrueckii* ssp. *Bulgaricus,* and *Lactococcus lactis* ssp. *cremoris,* have been reported for their effective action to produce bioactive peptides or hydrolysates from natural food sources (109). In addition to live microbes, lactic acid-bacteria-derived proteolytic enzymes have also been successfully used for protein hydrolysis to produce bioactive peptides. Although microbial fermentation is mainly relevant to the production of peptides from dairy products, it has been shown that fermentation can also produce bioactive peptides from beans, wheat, rice, and soy (110–113).

Enzymatic hydrolysis is a more efficient, safe, and reliable method than microbial fermentation as it takes less reaction time and is easy to control; additionally, it can be used to improve the biological and functional properties of proteins (114). These enzymes catalyze the hydrolysis of peptides bonds and may act on ester or amide bonds. All proteases have a certain degree of specificity in their substrate, generally based on a sequence of amino acids directly surrounding the bond that is cleaved (115). An extensive variety of bioactive hydrolysates and peptides have been produced from peanut, corn, soy, whey, and other protein sources (116–119). A conventional approach is mostly used to produce and identify the bioactive peptides (**Figure 4**). The efficacy of hydrolysates or peptides strongly depends upon the protein source, pretreatment of protein, proteases, and other hydrolysis factors, such as time, pH, and temperature (120). Proteases mainly involve two groups of enzymes i.e., exopeptidase (acts on amino or carboxyl ends of protein or peptides) and endopeptidase (acts on the interior of protein sequence) to break the peptide bonds (121). Most of the commercially available enzymes used for the production

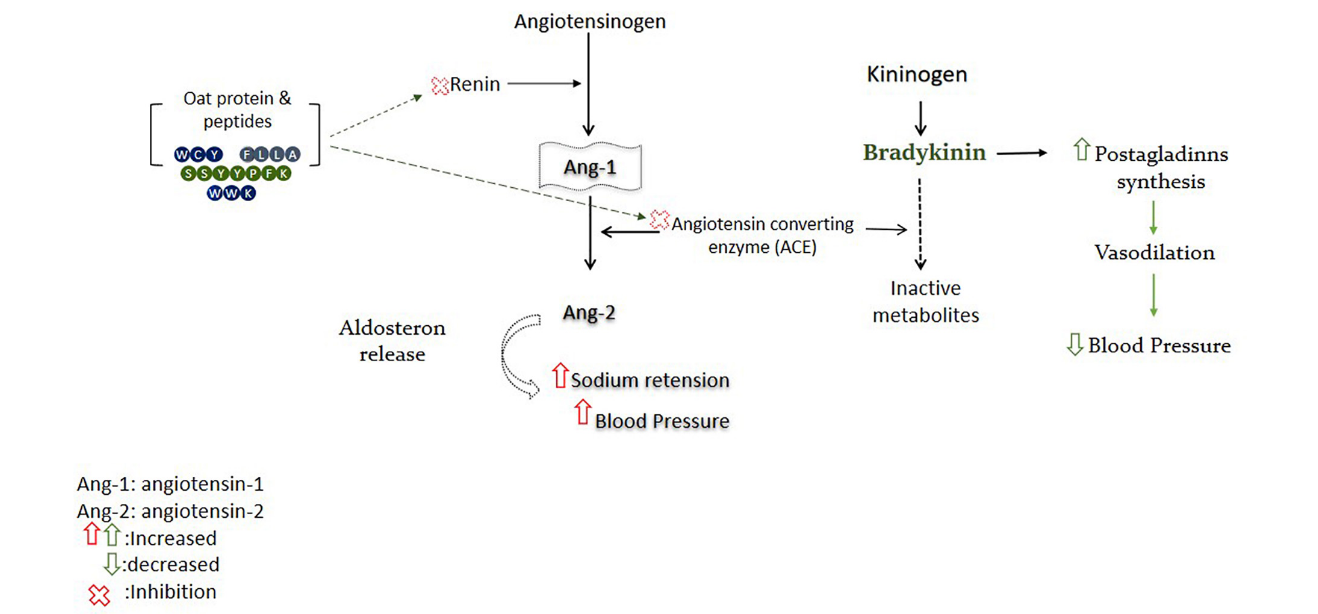

FIGURE 3 | Oat-derived bioactive peptides improve blood pressure by targeting RAS.

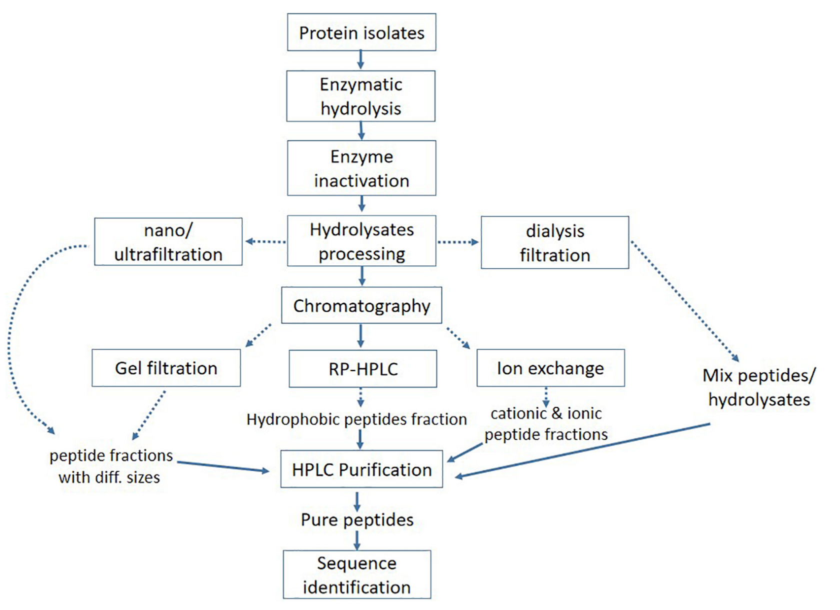

FIGURE 4 | Conventional approach for processing and purification of bioactive peptides.

of bioactive peptides are derived from animals (e.g., pepsin or trypsin), microbial (e.g., Alcalase, Flavourzyme, and Neutrase), and plants (bromelain and papain) origin (122). Apart from commercial enzymes, some studies reported the crude enzymes for protein hydrolysis, suggesting the potential application of novel proteases source to produce bioactive peptides.

Several physical techniques, such as ultrasound, microwave, and high pressure, have been reported to show favorable effects on increased hydrolysis and release of potent bioactive peptides from precursor protein (123). Utilizing these cell disruptive green extraction techniques has proved to be more effective in protein recovery with minimum environmental pollution and also improves the yield, functional, and nutritional properties of proteins (124). The Ultrasonic waves can disrupt the food matrix and facilitate the extraction of protein (125). Ultrasound treatment can also affect the secondary structure of proteins, which can alter their behavior during enzymatic hydrolysis and consequently, improve the biological activities of hydrolysates.

Numerous studies have shown the favorable effects of ultrasound on protein isolates of oat, corn, sunflower, rapeseed, and whey protein, as it helped in producing more active hydrolysates and short peptides, as well as improved the physicochemical and functional properties of protein isolates (63, 126–129). The microwave was shown to aid in chia seed proteolysis with improved bioactivity (antioxidant activity) and functionality (foaming and emulsifying properties) in a shorter time than simple enzymatic hydrolysis (130). Similarly, a pulsed electric field can enhance the antioxidant capacity of pine nuts by changing the secondary and tertiary structure of pentapeptide and protein (131). In addition to the above-mentioned isolation methods, chemical synthesis has also been used to obtain antioxidant, DPP4, and ACE inhibitory bioactive peptides (47, 64, 62). Most of the oat protein-derived bioactive peptides and hydrolysates discussed in this review have been produced using microbial and plant-based enzymes, including Alcalase, flavourzyme, and papain, while some peptides are produced by using gastrointestinal enzymes (trypsin and pepsin) as they mimic normal human digestion (48, 56, 94). However, enzyme–substrate ratio, degree of hydrolysis, and hydrolysis time are the important factors that need to be considered during the enzymatic hydrolysis process.

Purification and Identification of Bioactive Peptides

Enzymatic hydrolysates need appropriate separation and purification to evaluate the structure–activity relationship as it contains a mixture of several bioactive peptides, unhydrolyzed protein, and polypeptides of different lengths among others. To evaluate the accurate structure–activity relationship, various separation and purification techniques, including membrane separation, size-exclusion chromatography, HPLC, UPLC, and RP-HPLC, are widely used to get purified bioactive peptides (132). Ultra-high pressure chromatography (UPLC) is most suitable to purify the small-sized bioactive peptides. The main advantages of UPLC include increased resolution, throughput, and sensitivity (133). Reverse-phase chromatography (RP-HPLC) is used to separate the peptides based on hydrophobicity (134). The hydrophilic interaction liquid chromatography (HILIC) is a useful technique to separate the hydrophilic peptides (135). In addition, HILIC is a valuable tool to improve the separation of short peptides and the differentiation of homologous sequence peptides through mass spectrometry (136). Membrane separations or ultrafiltration and gel electrophoresis have also been used as subsidiary approaches for the chemical or structural configuration of peptides (132, 137). Similarly, electrodialysis with filtration membrane (EDFM) could fractionate the active peptides from complex hydrolysates on a molecular charge and mass basis (138, 139). After a series of isolation and purification procedures, the peptide's structure, and composition need to be identified. It is worth noting that mass spectrometry has greatly improved the process of studying protein profiles or hydrolysis products and identifying peptides' sequence. Liquid chromatography mass-spectrometry (LC-MS) is most commonly used in the identification of peptides sequence due to the advantages of high efficacy, sensitivity, and good reproducibility (140, 141).

Peptides with antioxidant activity that were obtained from oat globulin by using alcalase were initially separated and purified with chromatography, and the most active fraction was applied to ESI-MS/MS to identify the sequence as FNDILRRGQLL, IRIPL, FLKPNIT, NSKNFPTL, and LIGRPIIY (54). Similarly, antidiabetic peptides that were also obtained from the hydrolysis of oat globulin with trypsin were passed through an ultrafiltration membrane and further purified on gel chromatography, and the peptides elute was applied to Nano-LC-ESI-MS/MS for sequence identification. Two highly active antidiabetic peptides were identified as LQAFEPLR and EFLLAGNNK (61). Although ultra-filtration can significantly increase the bioactivity of peptides, this technique is not enough to get a highly pure product. Besides, the membrane is easily blocked by raw material, which causes pollution and the waste of raw material. Different techniques have some advantages and disadvantages, which make it difficult to obtain ideal peptides by using only a single technology. Therefore, the combination of separation techniques can attain precise classification and separation of peptides mixture to obtain high pure peptides.

CONCLUSION AND RECOMMENDATION

In summary, oats protein with higher content, unique amino acid composition, and less environmental impact in sense of land use, GHG emissions, and carbon footprint is a potential candidate for developing plant protein-based functional products. Oat protein with no allergic characteristics is likely to play a significant role in the meat alternative market over pea and soy proteins. It can be used to incorporate as a functional ingredient in various food products or to produce improved quality yogurt. Besides, the health benefits of oat protein and peptides make these compounds nutraceutical food additives in the formulation of functional foods. Enzymatic hydrolysis is a more efficient and reliable method to produce active peptides. Oat peptides have been shown to have remarkable biological activities by targeting specific molecular pathways of various chronic disorders. These peptides are considered health improving and disease-preventing agents by functioning as an antidiabetic, antihypertensive, antioxidant, anti-hypoxia, anti-fatigue, antithrombotic, and anti-hypercholesterolemia among others. However, more research should be carried out to evaluate the bioavailability and interactions of oat peptides with other food components and body organs to determine their wellbeing for human consumption. It is also important to develop and implement strategies to confirm the valorization of the nutritional and functional potential of oat protein and peptides for their exploration in the development of marketable nutraceutical and functional foods.

AUTHOR CONTRIBUTIONS

HR collected the data and drafted the manuscript. RD and XW helped to design the study. AA constructed the figures. RA, LZ, and LL revised the manuscript. XH supervised, revised, and approved the final version for publication. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Guerrieri N, Cavaletto M. Cereals proteins. . In: Yada RY editor. *Proteins in Food Processing*. (Kidlington: Elsevier) (2018). p. 223–44. doi: 10.1111/j.1365-2621.1946.tb16361.x
2. FAO, Brief D. *World Food Situation*. Rome: Food and Agriculture Organization of the United Nations (2019).
3. Cui YF. *Oats Nutrition and Technology*. Illinois, United States: John Wiley & Sons (2014). p. 171–94.
4. Menon R, Gonzalez T, Ferruzzi M, Jackson E, Winderl D, Watson J. Oats—from farm to fork. *Adv Food Nutr Res.* (2016) 77:1–55. doi: 10.1016/bs.afnr.2015.12.001
5. Biel W, Jacyno E, Kawęcka M. Chemical composition of hulled, dehulled and naked oat grains. *S Afr J Anim Sci.* (2014) 44:189–97.
6. Beloshapka AN, Buff PR, Fahey GC, Swanson KS. Compositional analysis of whole grains, processed grains, grain co-products, and other carbohydrate sources with applicability to pet animal nutrition. *Foods.* (2016) 5:23. doi: 10.3390/foods5020023
7. Boukid F, Zannini E, Carini E, Vittadini E. Pulses for bread fortification: a necessity or a choice? *Trends Food Sci Technol.* (2019) 88:416–28.
8. Wijewardana C, Reddy KR, Bellaloui N. Soybean seed physiology, quality, and chemical composition under soil moisture stress. *Food Chem.* (2019) 278:92–100. doi: 10.1016/j.foodchem.2018.11.035
9. de Oliveira Silva F, Miranda TG, Justo T, da Silva Frasão B, Conte-Junior CA, Monteiro M. Soybean meal and fermented soybean meal as functional ingredients for the production of low-carb, high-protein, high-fiber and high isoflavones biscuits. *LWT.* (2018) 90:224–31.
10. Majid A, Priyadarshini CGP. Millet derived bioactive peptides: a review on their functional properties and health benefits. *Crit Rev Food Sci Nutr.* (2020) 60:3342–51. doi: 10.1080/10408398.2019.1686342
11. Žilić S, Barać M, Pešić M, Dodig D, Ignjatović-Micić D. Characterization of proteins from grain of different bread and durum wheat genotypes. *Int J Mol Sci.* (2011) 12:5878–94. doi: 10.3390/ijms12095878
12. Nivala O, Mäkinen OE, Kruus K, Nordlund E, Ercili-Cura D. Structuring colloidal oat and FABA bean protein particles *via* enzymatic modification. *Food Chem.* (2017) 231:87–95. doi: 10.1016/j.foodchem.2017.03.114
13. Zhang B, Guo X, Zhu K, Peng W, Zhou H. Improvement of emulsifying properties of oat protein isolate–dextran conjugates by glycation. *Carbohydr Polym.* (2015) 127:168–75. doi: 10.1016/j.carbpol.2015.03.072
14. Amagliani L, O'Regan J, Kelly AL, O'Mahony JA. Composition and protein profile analysis of rice protein ingredients. *J Food Compos Anal.* (2017) 59:18–26.
15. Monteiro PV, Sudharshana L, Ramachandra G. Japanese barnyard millet (*Echinochloa frumentacea*): protein content, quality and SDS–PAGE of protein fractions. *J Sci Food Agric.* (1988) 43:17–25.
16. Adebiyi AP, Aluko RE. Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. *Food Chem.* (2011) 128:902–8.
17. Graveland A, Bosveld P, Lichtendonk WJ, Moonen HEH, Scheepstra A. Extraction and fractionation of wheat flour proteins. *J Sci Food Agric.* (1982) 33:1117–28.
18. Nieto-Nieto TV, Wang YX, Ozimek L, Chen L. Inulin at low concentrations significantly improves the gelling properties of oat protein – a molecular mechanism study. *Food Hydrocoll.* (2015) 50:116–27.
19. Yang C, Wang Y, Chen L. Fabrication, characterization and controlled release properties of oat protein gels with percolating structure induced by cold gelation. *Food Hydrocoll.* (2017) 62:21–34.
20. Jing X, Yang C, Zhang L. Characterization and analysis of protein structures in oat bran. *J Food Sci.* (2016) 81:C2337–43. doi: 10.1111/1750-3841.13445
21. Anderson OD. The spectrum of major seed storage genes and proteins in oats (*Avena sativa*). *PLoS One.* (2014) 9:e83569. doi: 10.1371/journal.pone.0083569
22. World Health Organization, United Nations University. *Protein and Amino acid Requirements in Human Nutrition*. Geneva: World Health Organization (2007). p. 1–265.
23. Walters ME, Udenigwe CC, Tsopmo A. Structural characterization and functional properties of proteins from oat milling fractions. *J Am Oil Chem Soc.* (2018) 95:991–1000.
24. Brückner-Gühmann M, Banovic M, Drusch S. Towards an increased plant protein intake: rheological properties, sensory perception and consumer acceptability of lactic acid fermented, oat-based gels. *Food Hydrocoll.* (2019) 96:201–8.
25. Van Zanten HHE, Herrero M, Van Hal O, Röös E, Muller A, Garnett T. Defining a land boundary for sustainable livestock consumption. *Glob Chang Biol.* (2018) 24:4185–94. doi: 10.1111/gcb.14321
26. Poore J, Nemecek T. Reducing food's environmental impacts through producers and consumers. *Science.* (2018) 360:987–92.
27. Shepon A, Eshel G, Noor E, Milo R. Energy and protein feed-to-food conversion efficiencies in the US and potential food security gains from dietary changes. *Environ Res Lett.* (2016) 11:105002.
28. Heusala H, Sinkko T, Mogensen L, Knudsen MT. Carbon footprint and land use of food products containing oat protein concentrate. *J Clean Prod.* (2020) 276:122938.
29. Carlsson-Kanyama A, González AD. Potential contributions of food consumption patterns to climate change. *Am J Clin Nutr.* (2009) 89:1704–9. doi: 10.3945/ajcn.2009.26736AA
30. Xue X, Landis AE. Eutrophication potential of food consumption patterns. *Environ Sci Technol.* (2010) 44:6450–6. doi: 10.1021/es9034478
31. Nijdam D, Rood T, Westhoek H. The price of protein: review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. *Food Policy.* (2012) 37:760–70.
32. Heusala H, Sinkko T, Sözer N, Hytönen E, Mogensen L, Knudsen MT. Carbon footprint and land use of oat and faba bean protein concentrates using a life cycle assessment approach. *J Clean Prod.* (2020) 242:1–9.
33. Mogensen L, Heusale H, Sinkko T, Poutanen K, Sözer N, Hermansen JE. Potential to reduce GHG emissions and land use by substituting animal-based proteins by foods containing oat protein concentrate. *J Clean Prod.* (2020) 10:122914.
34. Röös E, Patel M, Spångberg J. Producing oat drink or cow's milk on a Swedish farm – environmental impacts considering the service of grazing, the opportunity cost of land and the demand for beef and protein. *Agric Syst.* (2016) 142:23–32.
35. Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB. Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids.* (2018) 50:1685–95. doi: 10.1007/s00726-018-2640-5
36. Sarmadi BH, Ismail A. Antioxidative peptides from food proteins: a review. *Peptides.* (2010) 31:1949–56. doi: 10.1016/j.peptides.2010.06.020

37. Chalamaiah M, Ulug SK, Hong H, Wu J. Regulatory requirements of bioactive peptides (protein hydrolysates) from food proteins. *J Funct Foods.* (2019) 58:123–9.
38. Rabail R, Khan MR, Mehwish HM, Rajoka MSR, Lorenzo JM, Kieliszek M. An overview of chia seed (*Salvia hispanica* L.) bioactive peptides' derivation and utilization as an emerging nutraceutical food. *Front Biosci Landmark.* (2021) 26:643–54. doi: 10.52586/4973
39. Jiang N, Zhang S, Zhu J, Shang J, Gao X. Hypoglycemic, hypolipidemic and antioxidant effects of peptides from red deer antlers in streptozotocin-induced diabetic mice. *Tohoku J Exp Med.* (2015) 236:71–9. doi: 10.1620/tjem.236.71
40. Boukid F. Plant-based meat analogues: from niche to mainstream. *Eur Food Res Technol.* (2021) 247:297–308.
41. Shah A, Masoodi FA, Gani A, Ashwar BA. Newly released oat varieties of himalayan region –techno-functional, rheological, and nutraceutical properties of flour. *LWT.* (2016) 70:111–8.
42. 360 Market Updates. *Global Oat Protein Market Insighs, Forecast to 2025*. Pune: 360 Market Updates (2018).
43. Mel R, Malalgoda M. Oat protein as a novel protein ingredient: structure, functionality, and functionality, and factors impacting utilization. *Cereal Chem.* (2022) 99:21–36.
44. Boukid F. Oat proteins as emerging ingredients for food formulation: where we stand? *Eur Food Res Technol.* (2021) 247:535–44.
45. Mäkinen OE, Sozer N, Ercili-Cura D, Poutanen K. Protein from oat: structure, processes, functionality, and nutrition. In: Nadathur SR, Wanasundra JPD, Scanlin L editors. *Sustainable Protein Sources*. (Cambridge, MA: Academic Press) (2017). p. 105–19.
46. Brückner-Gühmann M, Benthin A, Drusch S. Enrichment of yoghurt with oat protein fractions: structure formation, textural properties and sensory evaluation. *Food Hydrocoll.* (2019) 86:146–53.
47. Kyriakopoulou K, Dekkers B, van der Goot AJ. Plant-based meat analogues. In: Galanakis CM editor. *Sustainable meat Production and Processing*. (London: Academic Press) (2019). p. 103–26.
48. de Angelis D, Kaleda A, Pasqualone A, Vaikma H, Tamm M, Tammik ML. Physicochemical and sensorial evaluation of meat analogues produced from dry-fractionated pea and oat proteins. *Foods.* (2020) 9:1754. doi: 10.3390/foods9121754
49. Grand View Research. *Oat milk Market size, Share and Trends Analysis Report by Product (Plain, Flavored), by Source (Organic, Conventional), by Application (Food, Beverages), by Region (Europe, APAC, North America), and Segment Forecasts, 2020-2027*. Pune: Grand View Research (2020).
50. Peterson DM. Oat antioxidants. *J Cereal Sci.* (2001) 33:115–29. doi: 10.1021/jf011222z
51. Chen J, Hu Y, Wang J, Hu H, Cui H. Combined effect of ozone treatment and modified atmosphere packaging on antioxidant defense system of fresh-cut green peppers. *J Food Process Preserv.* (2016) 40:1145–50.
52. Baakdah MM, Tsopmo A. Identification of peptides, metal binding and lipid peroxidation activities of HPLC fractions of hydrolyzed oat bran proteins. *J Food Sci Technol.* (2016) 53:3593–601. doi: 10.1007/s13197-016-2341-6
53. Esfandi R, Willmore WG, Tsopmo A. Peptidomic analysis of hydrolyzed oat bran proteins, and their *in vitro* antioxidant and metal chelating properties. *Food Chem.* (2019) 279:49–57.
54. Ma S, Zhang M, Bao X, Fu Y. Preparation of antioxidant peptides from oat globulin. *CyTA J Food.* (2020) 18:108–15.
55. Du Y, Esfandi R, Willmore WG, Tsopmo A. Antioxidant activity of oat proteins derived peptides in stressed hepatic HepG2 cells. *Antioxidants.* (2016) 5:39. doi: 10.3390/antiox5040039
56. Esfandi R, Willmore WG, Tsopmo A. Antioxidant and anti-apoptotic properties of oat bran protein hydrolysates in stressed hepatic cells. *Foods.* (2019) 8:160. doi: 10.3390/foods8050160
57. Esfandi R, Seidu I, Willmore W, Tsopmo A. Antioxidant, pancreatic lipase, and α−amylase inhibitory properties of oat bran hydrolyzed proteins and peptides. *J Food Biochem.* (2021) 46:e13762. doi: 10.1111/jfbc.13762
58. He YQ, Yang PY, Ding YY, Chen M, Guo R, Duan YQ. The preparation, antioxidant activity evaluation, and iron-deficient anemic improvement of oat (*Avena sativa* L.) peptides–ferrous chelate. *Front Nutr.* (2021) 8:687133. doi: 10.3389/fnut.2021.687133
59. Feng B, Ma L, Yao J, Fang Y, Mei Y, Wei S. Protective effect of oat bran extracts on human dermal fibroblast injury induced by hydrogen peroxide. *J Zhejiang Univ Sci B.* (2013) 14:97–105. doi: 10.1631/jzus.B1200159
60. Chakrabarti S, Jahandideh F, Davidge ST, Wu J. Milk-derived tripeptides IPP (Ile-Pro-Pro) and VPP (Val-Pro-Pro) enhance insulin sensitivity and prevent insulin resistance in 3T3-F442A preadipocytes. *J Agric Food Chem.* (2018) 66:10179–87. doi: 10.1021/acs.jafc.8b02051
61. Wang F, Zhang Y, Yu T, He J, Cui J, Wang J. Oat globulin peptides regulate antidiabetic drug targets and glucose transporters in Caco-2 cells. *J Funct Foods.* (2018) 42:12–20.
62. Wang TY, Hsieh CH, Hung CC, Jao CL, Chen MC, Hsu KC. Fish skin gelatin hydrolysates as dipeptidyl peptidase IV inhibitors and glucagon-like peptide-1 stimulators improve glycaemic control in diabetic rats: a comparison between warm-and cold-water fish. *J Funct Foods.* (2015) 19:330–40.
63. Walters ME, Willmore WG, Tsopmo A. Antioxidant, physicochemical, and cellular secretion of glucagon-like peptide-1 properties of oat bran protein hydrolysates. *Antioxidants.* (2020) 9:557. doi: 10.3390/antiox9060557
64. Payan F. Structural basis for the inhibition of mammalian and insect α-amylases by plant protein inhibitors. *Biochim Biophys Acta.* (2004) 1696:171–80. doi: 10.1016/j.bbapap.2003.10.012
65. Kim K, Park M, Lee YM, Rhyu MR, Kim HY. Ginsenoside metabolite compound K stimulates glucagon-like peptide-1 secretion in NCI-H716 cells *via* bile acid receptor activation. *Arch Pharm Res.* (2014) 37:1193–200. doi: 10.1007/s12272-014-0362-0
66. Wang JB, Liu XR, Liu SQ, Mao RX, Hou C, Zhu N, et al. Hypoglycemic effects of oat oligopeptides in high-calorie diet/STZ-induced diabetic rats. *Molecules.* (2019) 24:558. doi: 10.3390/molecules24030558
67. Rytter E, Erlanson-Albertsson C, Lindahl L, Lundquist I, Viberg U, Åkesson B. Changes in plasma insulin, enterostatin, and lipoprotein levels during an energy-restricted dietary regimen including a new oat-based liquid food. *Ann Nutr Metab.* (1996) 40:212–20. doi: 10.1159/000177921
68. Pino JL, Mujica V, Arredondo M. Effect of dietary supplementation with oat β-glucan for 3 months in subjects with type 2 diabetes: a randomized, double-blind, controlled clinical trial. *J Funct Foods.* (2021) 77:104311.
69. Fisher NDL, Hollenberg NK. Renin inhibition: what are the therapeutic opportunities? *J Am Soc Nephrol.* (2005) 16:592–9.
70. Bleakley S, Hayes M, O'Shea N, Gallagher E, Lafarga T. Predicted release and analysis of novel ACE-I, renin, and DPP-IV inhibitory peptides from common oat (*Avena sativa*) protein hydrolysates using in silico analysis. *Foods.* (2017) 6:108. doi: 10.3390/foods6120108
71. Hong S, Cheung HS, Wang F, Ma O, Ef S. Binding of peptide substrates and inhibitors of angiotensin-converting enzyme: importance of the COOH-terminal dipeptide sequence. *J Biol Chem.* (1980) 255:401–7.
72. Zheng Y, Wang X, Zhuang Y, Li Y, Shi P, Tian H. Isolation of novel ACE-inhibitory peptide from naked oat globulin hydrolysates in silico approach: molecular docking, *in vivo* antihypertension and effects on renin and intracellular endothelin-1. *J Food Sci.* (2020) 85:1328–37. doi: 10.1111/1750-3841.15115
73. Zhao YQ, Zhang L, Tao J, Chi CF, Wang B. Eight antihypertensive peptides from the protein hydrolysate of antarctic krill (*Euphausia superba*): isolation, identification, and activity evaluation on human umbilical vein endothelial cells (HUVECs). *Food Res Int.* (2019) 121:197–204. doi: 10.1016/j.foodres.2019.03.035
74. Bouchard J, Valookaran AF, Aloud BM, Raj P, Malunga LN, Thandapilly SJ. Impact of oats in the prevention/management of hypertension. *Food Chem.* (2022) 381:132198. doi: 10.1016/j.foodchem.2022.132198
75. Mao R, Wu L, Zhu N, Liu X, Liu R, Li Y. Naked oat (*Avena nuda* L.) oligopeptides: immunomodulatory effects on innate and adaptive immunity in mice *via* cytokine secretion, antibody production, and Th cells stimulation. *Nutrients.* (2019) 11:927. doi: 10.3390/nu11040927
76. Constant SL, Bottomly K. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu Rev Immunol.* (1997) 15:297–322. doi: 10.1146/annurev.immunol.15.1.297
77. Tang Y, Li S, Yan J, Peng Y, Weng W, Yao X, et al. Bioactive components and health functions of oat bioactive components and health functions of oat. *Food Rev Int.* (2022) 133:1–20. doi: 10.1080/87559129.2022.2029477

78. Gao X, Zhang H, Zhuang W, Yuan G, Sun T, Jiang X. PEDF and PEDF-derived peptide 44mer protect cardiomyocytes against hypoxia-induced apoptosis and necroptosis *via* anti-oxidative effect. *Sci Rep.* (2014) 4:1–7. doi: 10.1038/srep05637
79. Lee CS, Choi EY, Lee SC, Koh HJ, Lee JH, Chung JH. Resveratrol inhibits hypoxia-induced vascular endothelial growth factor expression and pathological neovascularization. *Yonsei Med J.* (2015) 56:1678–85. doi: 10.3349/ymj.2015.56.6.1678
80. Wang J, Li LZ, Liu YG, Teng LR, Lu JH, Xie J. Investigations on the antifatigue and antihypoxic effects of *Paecilomyces hepiali* extract. *Mol Med Rep.* (2016) 13:1861–8. doi: 10.3892/mmr.2015.4734
81. Di Li, Ren J, Du Q, Liu P, Li Y. The anti-hypoxic effects of oat (*Avena sativa* L.) oligopeptides in mice. *Am J Transl Res.* (2021) 13:1657.
82. Kehrer JP, Lund LG. Cellular reducing equivalents and oxidative stress. *Free Radic Biol Med.* (1994) 17:65–75. doi: 10.1016/0891-5849(94)90008-6
83. Shohami E, Beit-Yannai E, Horowitz M, Kohen R. Oxidative stress in closed-head injury: brain antioxidant capacity as an indicator of functional outcome. *J Cereb Blood Flow Metab.* (1997) 17:1007–19. doi: 10.1097/00004647-199710000-00002
84. Halliwell B. Role of free radicals in the neurodegenerative diseases. *Drugs Aging.* (2001) 18:685–716. doi: 10.2165/00002512-200118090-00004
85. Rauchova H, Vokurkova M, Koudelova J. Hypoxia-induced lipid peroxidation in the brain during postnatal ontogenesis. *Physiol Res.* (2012) 61:S89. doi: 10.33549/physiolres.932374
86. Zhu N, Mao R, Liu R. Anti-fatigue effect of oat peptide in mice: an experimental study. *Chinese J Public Health.* (2018) 34:1242–5.
87. Surenkok O, Kin-Isler A, Aytar A, Gültekin Z. Effect of trunk-muscle fatigue and lactic acid accumulation on balance in healthy subjects. *J Sport Rehabil.* (2008) 17:380–6. doi: 10.1123/jsr.17.4.380
88. Xu C, Lv J, You S, Zhao Q, Chen X, Hu X. Supplementation with oat protein ameliorates exercise-induced fatigue in mice. *Food Funct.* (2013) 4:303–9. doi: 10.1039/c2fo30255a
89. Sahlin K, Sallstedt EK, Bishop D, Tonkonogi M. Turning down lipid oxidation during heavy exercise—what is the mechanism. *J Physiol Pharmacol.* (2008) 59:19–30.
90. Jia JM, Wu CF. Antifatigue activity of tissue culture extracts of *Saussurea involucrata*. *Pharm Biol.* (2008) 46:433–6.
91. You L, Zhao M, Regenstein JM, Ren J. *In vitro* antioxidant activity and *in vivo* anti-fatigue effect of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. *Food Chem.* (2011) 124:188–94.
92. Ding JF, Li YY, Xu JJ, Su XR, Gao X, Yue FP. Study on effect of jellyfish collagen hydrolysate on anti-fatigue and anti-oxidation. *Food Hydrocoll.* (2011) 25:1350–3.
93. Zhang Y, Yao X, Bao B, Zhang Y. Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (Caulis bamfusae in taeniam). *Phyther Res.* (2006) 20:872–6. doi: 10.1002/ptr.1965
94. Coombes JS, McNaughton LS. Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. *J Sports Med Phys Fitness.* (2000) 40:240.
95. Xia Z, Cholewa JM, Dardevet D, Huang T, Zhao Y, Shang H. Effects of oat protein supplementation on skeletal muscle damage, inflammation and performance recovery following downhill running in untrained collegiate men. *Food Funct.* (2018) 9:4720–9. doi: 10.1039/c8fo00786a
96. Jackson SP, Schoenwaelder SM. Antiplatelet therapy: in search of the'magic bullet'. *Nat Rev Drug Discov.* (2003) 2:775–89. doi: 10.1038/nrd1198
97. Wang XH, Shao DH, Liang GW, Zhang R, Xin Q, Zhang T. Cyclooxygenase inhibitors in some dietary vegetables inhibit platelet aggregation function induced by arachidonic acid. *J Exp Hematol.* (2011) 19:1260–3.
98. Lee DH, Kim YJ, Kim HH, Cho HJ, Ryu JH, Rhee MH. Inhibitory effects of epigallocatechin-3-gallate on microsomal cyclooxygenase-1 activity in platelets. *Biomol Ther (Seoul).* (2013) 21:54. doi: 10.4062/biomolther.2012.075
99. Oder E, Safo MK, Abdulmalik O, Kato GJ. New developments in anti-sickling agents: can drugs directly prevent the polymerization of sickle haemoglobin *in vivo*? *Br J Haematol.* (2016) 175:24–30. doi: 10.1111/bjh.14264
100. Yu G, Wang F, Zhang B, Fan J. *In vitro* inhibition of platelet aggregation by peptides derived from oat (*Avena sativa* L.), highland barley (*Hordeum vulgare* Linn. var. nudum Hook. f.), and buckwheat (*Fagopyrum esculentum* Moench) proteins. *Food Chem.* (2016) 194:577–86. doi: 10.1016/j.foodchem.2015.08.058
101. Connolly ML, Tzounis X, Tuohy KM, Lovegrove JA. Hypocholesterolemic and prebiotic effects of a whole-grain oat-based granola breakfast cereal in a cardio-metabolic "at risk" population. *Front Microbiol.* (2016) 7:1675. doi: 10.3389/fmicb.2016.01675
102. Guo L, Tong LT, Liu L, Zhong K, Qiu J, Zhou S. The cholesterol-lowering effects of oat varieties based on their difference in the composition of proteins and lipids. *Lipids Health Dis.* (2014) 13:1–10. doi: 10.1186/1476-511X-13-182
103. Tong L, Guo L, Zhou X, Qiu J, Liu L, Zhong K, et al. Effects of dietary oat proteins on cholesterol metabolism of hypercholesterolaemic hamsters. *J Sci Food Agric.* (2016) 96:1396–401. doi: 10.1002/jsfa.7236
104. Daou C, Zhang H. Oat beta-glucan: its role in health promotion and prevention of diseases. *Compr Rev Food Sci Food Saf.* (2012) 11:355–65.
105. Maki KC, Shinnick F, Seeley MA, Veith PE, Quinn LC, Hallissey PJ. Food products containing free tall oil-based phytosterols and oat β-glucan lower serum total and LDL cholesterol in hypercholesterolemic adults. *J Nutr.* (2003) 133:808–13. doi: 10.1093/jn/133.3.808
106. Anderson JW, Gilinsky NH, Deakins DA, Smith SF, O'Neal DS, Dillon DW, et al. Lipid responses of hypercholesterolemia men to oat-bran and wheat-bran intake. *Am J Clin Nutr.* (1991) 54:678–83. doi: 10.1093/ajcn/54.4.678
107. Reyna-Villasmil N, Bermúdez-Pirela V, Mengual-Moreno E, Arias N, Cano-Ponce C, Leal-Gonzalez E. Oat-derived β-glucan significantly improves HDLC and diminishes LDLC and non-HDL cholesterol in overweight individuals with mild hypercholesterolemia. *Am J Ther.* (2007) 14:203–12. doi: 10.1097/01.pap.0000249917.96509.e7
108. Sánchez A, Vázquez A. Bioactive peptides: a review. *Food Qual Saf.* (2017) 1:29–46.
109. Hernández-Ledesma B, del Mar Contreras M, Recio I. Antihypertensive peptides: production, bioavailability and incorporation into foods. *Adv Colloid Interface Sci.* (2011) 165:23–35. doi: 10.1016/j.cis.2010.11.001
110. Singh BP, Vij S, Hati S. Functional significance of bioactive peptides derived from soybean. *Peptides.* (2014) 54:171–9. doi: 10.1016/j.peptides.2014.01.022
111. Inoue K, Gotou T, Kitajima H, Mizuno S, Nakazawa T, Yamamoto N. Release of antihypertensive peptides in miso paste during its fermentation, by the addition of casein. *J Biosci Bioeng.* (2009) 108:111–5. doi: 10.1016/j.jbiosc.2009.03.007
112. Limón RI, Peñas E, Torino MI, Martínez-Villaluenga C, Dueñas M, Frias J. Fermentation enhances the content of bioactive compounds in kidney bean extracts. *Food Chem.* (2015) 172:343–52. doi: 10.1016/j.foodchem.2014.09.084
113. Nakahara T, Sano A, Yamaguchi H, Sugimoto K, Chikata H, Kinoshita E. Antihypertensive effect of peptide-enriched soy sauce-like seasoning and identification of its angiotensin I-converting enzyme inhibitory substances. *J Agric Food Chem.* (2010) 58:821–7. doi: 10.1021/jf903261h
114. Waseem M, Kumar S, Kumar A. *Bioactive Peptides*. Hauppauge, NY: Nova Science Publisher Inc. (2018).
115. Santos LF, Koblitz MGB. *Proteases Bioquímica de Alimentos*. Rio de Janeiro: Guanabara Koogan (2008). p. 78–103. .
116. Madureira AR, Tavares T, Gomes AMP, Pintado ME, Malcata FX. Invited review : physiological properties of bioactive peptides obtained from whey proteins. *J Dairy Sci.* (2010) 93:437–55. doi: 10.3168/jds.2009-2566
117. Shi A, Liu H, Liu L, Hu H, Wang Q, Adhikari B. Isolation, purification and molecular mechanism of a peanut protein-derived ACE-inhibitory peptide. *PLoS One.* (2014) 9:e111188. doi: 10.1371/journal.pone.0111188
118. Zhang Q, Tong X, Li Y, Wang H, Wang Z, Qi B. Purification and characterization of antioxidant peptides from alcalase-hydrolyzed soybean (*Glycine max* L.) hydrolysate and their cytoprotective effects in human intestinal Caco-2 cells. *J Agric Food Chem.* (2019) 67:5772–81. doi: 10.1021/acs.jafc.9b01235
119. Zhu B, He H, Hou T. A comprehensive review of corn protein-derived bioactive peptides : production, characterization, bioactivities, and transport pathways. *Compr Rev Food Sci Food Saf.* (2019) 18:329–45. doi: 10.1111/1541-4337.12411

120. Zarei M, Ebrahimpour A, Abdul-Hamid A, Anwar F, Saari N. Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants. *Int J Mol Sci.* (2012) 13:8097–111. doi: 10.3390/ijms13078097
121. Cooper JB. Aspartic proteinases in disease: a structural perspective. *Curr Drug Targets.* (2002) 3:155–73. doi: 10.2174/1389450024605382
122. Mazorra-Manzano MA, Ramírez-Suarez JC, Yada RY. Plant proteases for bioactive peptides release: a review. *Crit Rev Food Sci Nutr.* (2018) 58:2147–63. doi: 10.1080/10408398.2017.1308312
123. Daroit DJ, Brandelli A. *In vivo* bioactivities of food protein-derived peptides–a current review. *Curr Opin Food Sci.* (2021) 39:120–9.
124. Kumar M, Tomar M, Potkule J, Verma R, Punia S, Mahapatra A. Advances in the plant protein extraction: mechanism and recommendations. *Food Hydrocoll.* (2021) 115:106595.
125. Zhu Z, Zhu W, Yi J, Liu N, Cao Y, Lu J, et al. Effects of sonication on the physicochemical and functional properties of walnut protein isolate. *Food Res Int.* (2018) 106:853–61. doi: 10.1016/j.foodres.2018.01.060
126. Jin J, Ma H, Wang B, Yagoub Ael-G, Wang K, He R. Effects and mechanism of dual-frequency power ultrasound on the molecular weight distribution of corn gluten meal hydrolysates. *Ultrason Sonochem.* (2016) 30:44–51. doi: 10.1016/j.ultsonch.2015.11.021
127. Malik MA, Sharma HK, Saini CS. High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: effect on physicochemical and functional properties. *Ultrason Sonochem.* (2017) 39:511–9. doi: 10.1016/j.ultsonch.2017.05.026
128. Wali A, Ma H, Shahnawaz M, Hayat K, Xiaong J, Jing L. Impact of power ultrasound on antihypertensive activity, functional properties, and thermal stability of rapeseed protein hydrolysates. *J Chem.* (2017) 2017:1–11.
129. Wu Q, Zhang X, Jia J, Kuang C, Yang H. Effect of ultrasonic pretreatment on whey protein hydrolysis by alcalase: thermodynamic parameters, physicochemical properties and bioactivities. *Process Biochem.* (2018) 67:46–54.
130. Urbizo-Reyes U, San Martin-González MF, Garcia-Bravo J, López Malo Vigil A, Liceaga AM. Physicochemical characteristics of chia seed (*Salvia hispanica*) protein hydrolysates produced using ultrasonication followed by microwave-assisted hydrolysis. *Food Hydrocoll.* (2019) 97:105187.
131. Liang R, Cheng S, Wang X. Secondary structure changes induced by pulsed electric field affect antioxidant activity of pentapeptides from pine nut (*Pinus koraiensis*) protein. *Food Chem.* (2018) 254:170–84. doi: 10.1016/j.foodchem.2018.01.090
132. Wen C, Zhang J, Zhang H, Duan Y, Ma H. Plant protein-derived antioxidant peptides: isolation, identification, mechanism of action and application in food systems: a review. *Trends Food Sci Technol.* (2020) 105:308–22.
133. Everley RA, Croley TR. Ultra-performance liquid chromatography/mass spectrometry of intact proteins. *J Chromatogr A.* (2008) 1192:239–47. doi: 10.1016/j.chroma.2008.03.058
134. Pownall TL, Udenigwe CC, Aluko RE. Amino acid composition and antioxidant properties of pea seed (*Pisum sativum* L.) enzymatic protein hydrolysate fractions. *J Agric Food Chem.* (2010) 58:4712–8. doi: 10.1021/jf904456r
135. Yoshida T. Peptide separation by hydrophilic-interaction chromatography: a review. *J Biochem Biophys Methods.* (2004) 60:265–80. doi: 10.1016/j.jbbm.2004.01.006
136. Le Maux S, Nongonierma AB, FitzGerald RJ. Improved short peptide identification using HILIC–MS/MS: retention time prediction model based on the impact of amino acid position in the peptide sequence. *Food Chem.* (2015) 173:847–54. doi: 10.1016/j.foodchem.2014.10.104
137. Roblet C, Amiot J, Lavigne C, Marette A, Lessard M, Jean J. Screening of *in vitro* bioactivities of a soy protein hydrolysate separated by hollow fiber and spiral-wound ultrafiltration membranes. *Food Res Int.* (2012) 46:237–49.
138. Ketnawa S, Suwal S, Huang J, Liceaga AM. Original article Selective separation and characterisation of dual ACE and DPP-IV inhibitory peptides from rainbow trout (*Oncorhynchus mykiss*) protein hydrolysates. *Int J Food Sci Technol.* (2019) 54:1062–73.
139. Suwal S, Ketnawa S, Liceaga AM, Huang J. Electro-membrane fractionation of antioxidant peptides from protein hydrolysates of rainbow trout (*Oncorhynchus mykiss*) byproducts. *Innov Food Sci Emerg Technol.* (2018) 45:122–31.
140. Boja ES, Rodriguez H. Mass spectrometry-based targeted quantitative proteomics: achieving sensitive and reproducible detection of proteins. *Proteomics.* (2012) 12:1093–110. doi: 10.1002/pmic.201100387
141. del Mar Contreras M, Lopez-Exposito I, Hernandez-Ledesma B, Ramos M, Recio I. Application of mass spectrometry to the characterization and quantification of food-derived bioactive peptides. *J AOAC Int.* (2008) 91:981–94.

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The Effects of Food Taxes and Subsidies on Promoting Healthier Diets in Iranian Households

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Background and Aim: Price, as a key driver of food purchasing, has an important role in determining the consumer demand. This study is aimed to estimate the effect of food taxes and subsidies on purchasing patterns of Iranian households (HHs).

Methods: This study was performed in two phases. In phase one, a two-round Delphi study was conducted to determine and prioritize food-related fiscal policies; and in the second phase, using the Iranian Household Income and Expenditure Survey (HIES), we estimated an almost ideal demand system (AIDS) and simulated changes in purchases, nutrient intake, and consumer welfare under six different policy scenarios: (1) 20% subsidy on vegetables, (2) 20% subsidy on fruits, (3) 30% subsidy on legumes, (4) 25% tax on sugar and sweets, (5) 30% tax on sweetened beverages, and (6) 30% tax on hydrogenated oil and animal fats.

Results: The highest calorie reduction was detected in sugar and sweets tax, which has resulted in 949.67, 971.68, and 1,148.03 kilocalories decrease in energy intake per Adult Male Equivalent (AME) in all HHs, low-income HHs, and high-income HHs, respectively. In terms of welfare changes, high-income HHs will experience a lower change in welfare (−0.81 to 0.11%) relative to their income when compared with low-income HHs (−0.88 to 0.28%) due to fiscal policies.

Conclusion: Fiscal policies in Iran can be a potential way to improve dietary choices. The findings provide essential information for decision makers for the implementation of food-related fiscal policies.

Keywords: fiscal policy, taxes, almost ideal demand system, Iran, diet

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BACKGROUND

Non-communicable diseases (NCDs) have been the most important global health problem over the last decades and are responsible for almost 40 million deaths each year (equivalent to 70% of all deaths, globally). The relationship between NCDs and unhealthy foods and drinks' consumption is well known. It has been shown that the dietary factors contribute to almost 10% of the global burden of diseases (1). In response to the growing evidence on the causal relationship between an unhealthy diet and increased NCDs risk, food and nutrition experts suggest different strategies that can contribute toward a healthy community, such as promotion of nutrition literacy, controlling food products advertising, food labeling, and food-related fiscal policies through taxation or subsidies (2, 3).

Since the early 1980s, several countries have implemented taxes on unhealthy foods and beverages, for revenue purposes and to reduce demand for their consumption (4). Fiscal policies have also been recommended by the World Health Organization (WHO) as a proper way to discourage the consumption of unhealthy foods and beverages (5). Price, as a key driver of food purchasing, has an important role in determining the consumer demand. Previous studies in real-world environments indicated that people tend to reduce their consumption of unhealthy foods as prices increase (6). To guide policy-making, research has an important role in estimating the likely impact of such fiscal policies on changes in household (HH) diet. A large number of recent studies have concentrated on estimating the impact of price changes on the demand for certain food categories, such as sugar-sweetened beverages (SSBs) (7, 8), saturated fats (9), and fruit and vegetables (10, 11).

One criticism commonly stated about food-related fiscal policies is that HHs in low- and middle-income countries would pay a greater percentage of their income on food and evidence from high-income countries may not be directly applicable to middle- and low-income countries (12). Alagiyawanna et al. conducted a systematic review to study the different outcomes of fiscal policies in countries of different income classifications. The results of their study supported previous findings that fiscal policies can have an impact on healthy food consumption; it also highlights the lack of enough evidence in low- and middle-income countries in this regard (13).

In Iran, since 2012, the annual average rate of food inflation has been over 29% (14). Previous studies conducted in the country have mainly focused on price increases and their impact on changes in HH welfare (15–17). To the best of our knowledge, health-related targeted food taxes and subsidies have not been studied so far. Thus, the aim of this study was to estimate the effects of targeted food taxes and subsidies on the food purchasing patterns of Iranian HHs.

MATERIALS AND METHODS

This study was performed in two phases. In phase one, policy options for food taxation or subsidizing were prioritized; and in the second phase, the effects of pricing policy on different income groups were evaluated using the existing data.

Phase 1: Food Tax and Subsidy Scenarios

This qualitative study was conducted from May to November 2021 using the Delphi approach. A Delphi study was performed on a panel that consisted of Iranian researchers who had published on food-related fiscal policies, health managers, and other experts in health and nutrition policy-making. Twenty-seven participants took part in this two-round Delphi study. The general characteristics of the participants are presented in the **Supplementary Table 1**. In the first round, the main question asked through the Delphi phases was "considering health issues, what food items or groups are appropriate and have the highest priority to be taxed or subsidized in Iran?"

As an indicator of consensus, the quantitative summary [mean, standard deviation (SD), median, and mode] of responses was calculated. A second questionnaire was created with the policy options proposed by the participants. Following that, the questionnaire was sent to all participants in case they wanted to change their opinion regarding the quantitative information of each policy option. Eventually, according to the consensus (mode) of responses, three tax scenarios and three subsidy scenarios were selected, which were as follows: (1) 20% subsidy on vegetables, (2) 20% subsidy on fruits, (3) 30% subsidy on legumes, (4) 25% tax on sugar and sweets, (5) 30% tax on SSBs, and (6) 30% tax on hydrogenated oil and animal fats. In the second round, the panel was asked to rate policy options with regard to six factors (impact of policy option on health, chance of stability, implementation feasibility, implementation costs, acceptance by authorities, and acceptance by authorities society) on a 5-point Likert scale (1 = very low, 2 = low, 3 = moderate, 4 = high, and 5 = very high). Data were analyzed using basic descriptive statistical tests and expressed as mean and SD. In addition, the highest mean indicated an option has high priority.

Phase 2: Evaluating Food Price Policy Effect

In this phase of the study, data from Iran's Households Income and Expenditure Survey (HIES) that is being carried out annually by the Statistical Centre of Iran were utilized. HIES provides information on an HH's living conditions and income/expenditure patterns. In the present study, we used data from 1990 to 2021. The sample size in this study at the national level was 927,680 HHs. In order to examine the pricing policy effects on different income groups, we created three sub-samples using the tertile of the HH income distribution, where lower tertile refers to HHs with lower income and likewise for higher tertile.

Demand Model

The Almost Ideal Demand System (AIDS) of Deaton and Muellbauer (18) by the method of seemingly unrelated regressions (SURs) was applied to estimate demand parameters for food groups. The AIDS model allows us to predict the potential demand response to changes in prices. All estimations were carried out using Eviews Software version 10 and Microsoft Excel 2010. The model is specified as follows:

$$w_i = \alpha_i + \Sigma\gamma_{ij} lnp_j + \beta_i \ln(Mt_i/P) + t_i d$$

Where w_i is the food or beverage expenditure share for food or beverage group i; p_j is the unit value for food or beverage j; Mt_i is real HH income; d is the family size, and P is the stone price index, defined as follows:

$$lnP = \sum_{i=1}^{n} w_i + \ln p_i$$

Marshallian and Hicksian price elasticities of the demand for the food groups were calculated using the following equations:

$$\varepsilon = -\delta_{ij} + \frac{\gamma_{ij}}{w_i} - \beta_i(\frac{wj}{w_i})$$

$$\varepsilon = -\delta_{ij} + \frac{\gamma_{ij}}{w_i} + w_j$$

Where ε is the price elasticity of the food or beverage category, δ equals 1 if it is own-price elasticity and 0 if cross-price elasticity, w is the mean expenditure share of food or beverage, β_i is the estimated parameter of the log real income, and γ_{ij} is the estimated parameter associated to the unit value of the food or beverage category. Additionally, the welfare change to consumers is estimated using the compensating variation (CV) measure, reflecting the income an HH needs to receive in order to return to the original utility level after a price change. Hicksian elasticities were used to calculate the CV as an established approach in the literature (19):

$$\triangle lnc = \Sigma_i w_i \triangle lnp_i + 1/2 \Sigma_i \Sigma_j w_i \varepsilon_{ij} \triangle lnp_i \triangle lnp_j$$

Change in Dietary Pattern

The 2020–2021 HIES data were applied to estimate the impact of food-related fiscal policies on the percent changes in HH purchases and also the amount of calorie and nutrient intake. The total sample size was 37,557 HHs. We determined the change in monthly intake of foods (e.g., amount of fruits and vegetables) and nutrients (e.g., carbohydrates and saturated fat) for each tax and subsidy scenario, using price elasticity data. Since data on the HH food basket were collected for the HHs as the sampling units, they were converted into individual amounts. The estimates were calculated per Adult Male Equivalent (AME) unit per month. AME is the ratio of the energy requirement of an HH member of a particular age and gender to the energy requirement of an adult male aged 18–30 years with moderate physical activity as recommended by the Food and Agriculture Organization (FAO) and WHO (20). In the present study, the total AME of the HH was calculated; then, the amount of each food item was divided by the total AME of the HH. Since purchased food is partly wasted, the real amount of the consumed foods was estimated based on the FAO-recommended waste percentage for each food group in the consumption phase (21). Food analyses were performed using the Nutritionist IV software, and the AME of the energy and nutrient intake were calculated manually.

RESULTS

Table 1 shows the policy options prioritized by the expert panel. The panel suggested that subsidizing vegetables had the greatest impact on health (mean score = 4.18) and acceptance by society (3.85), with the greatest implementation cost (3.88). In regards to "implementation feasibility" and "acceptance by the authorities," the tax on sweetened beverages had the highest agreement. Furthermore, taxation of hydrogenated oil and animal fats was considered to have the highest chance of stability (3.37).

Table 2 presents the descriptive statistics for the all HHs, low-income HHs, and high-income HHs defined as the first and third tertiles of the HH annual income distribution, respectively. The average age of the HH heads age was 51.87 years, while 85% of the HHs were male-headed, and the average HH size was about 3.43 people. Moreover, it is noted that low-income HHs had a mean income of 24,129.42 thousand Rials per month, whereas the mean income of high-income HHs was 68,802.3 thousand Rials/month.

Table 3 provides descriptive statistics on the 11 food categories for both total HHs and sub-samples by HH income. We report unit value (in thousand Rials), purchase quantity [in kilograms or liters (kg or L)], and budget share (% of income). Cereals in all groups have the highest budget share and quantity. In addition, red meat and vegetables have the highest and lowest unit prices, respectively. It has been observed that high-income HHs purchase more on every food group (except hydrogenated oils and animal fats), pay higher unit values, and spend a smaller share of their budget than low-income HHs.

Estimated Marshallian own- and cross-price elasticities are presented in the **Supplementary Tables 2–4**. Based on the Marshallian elasticities, the variations in purchases due to each fiscal policy for each food group were computed. The percent changes for all food groups are reported in **Table 4**. According to the table, legumes are more price elastic as compared to other targeted food groups in all groups (−1, −0.97, and −1.08 for all HHs, low-income HHs, and high-income HHs, respectively). The strongest demand responses were observed in policy 3, which would lead to an overall purchase increase of 30.24, 29.37, and

TABLE 1 | The mean (SD) scores of the prioritization criteria for each policy.

		Impact on health	Chance of stability	Implementation feasibility	Implementation costs	Acceptance by the authority	Acceptance by the society
Subsidy scenarios	20% on vegetables	4.18 (0.78)	2.4 (0.84)	2.37 (0.83)	3.88 (1.01)	1.92 (0.78)	3.85 (0.98)
	20% on fruits	4 (0.73)	2.62 (0.88)	2.55 (1)	3.59 (1)	2.07 (0.67)	3.81 (0.78)
	30% on legumes	3.88 (0.75)	2.52 (1.04)	2.25 (0.81)	3.74 (0.94)	2.18 (0.87)	3.74 (1.2)
Tax scenarios	25% on sugar	3.88 (0.84)	2.88 (0.84)	3.55 (1.08)	2.77 (0.75)	3.18 (1.07)	2.66 (1)
	30% on sweetened beverages	3.66 (0.91)	2.62 (1)	3.96 (1.05)	2.4 (0.79)	3.33 (1.07)	2.81 (0.92)
	30% on hydrogenated oil and animal fats	3.7 (0.86)	3.37 (1.04)	3.55 (1.15)	2.77 (1.08)	2.96 (1.02)	2.25 (1.1)

A 5-point Likert scale (1 = very low to 5 = very high) was used for scoring. SD, standard deviation.

TABLE 2 | Household descriptive statistics.

	All HHs	Low income HHs	High income HHs
Household head's age	51.87 (15.28)	54.4 (16.88)	47.73 (13.35)
Household head's education (illiterate %)	20.35	32	9.37
Female household head (%)	14.7	21	4.2
Household size	3.43 (1.44)	3.29 (0.51)	3.95 (0.81)
Households income (thousand rials)	39710.16 (48835.92)	24129.42 (17417.82)	68802.3 (57439.2)

HHs, households. Standard deviations (SDs) are in parentheses.

32.47% of legumes in all, low-income HHs, and high-income HHs, respectively. Whereas the weakest responses were detected in policy 1, that as a result, the overall purchase of vegetables would increase by 7.49, 5.5, and 9.63%, respectively, for the all, low-income HHs, and high-income HHs.

Table 5 shows the percentage changes in HH purchases converted to calorie and nutrient intake per AME per month. All policies affect the amount of nutrients intake. For example, all subsidy policies increased energy and dietary fiber intake in all groups. The sweetened beverage tax produced 144.23, 450.04, and 977.94 kilocalories decrease in energy intake per AME in all, low-income HHs, and high-income HHs, respectively. The sugar and sweets tax gave a notable reduction in sugar intake (235.13, 292.35, and 301.17 g per AME per month in all, low-income HHs, and high-income HHs, respectively; **Table 5**).

A comparison of the welfare change (measured by CV) is presented in **Table 6** in both thousand Rials and as a percentage of HH income. The welfare benefits of subsidy policies (policy 1–3) for households were 254.52, 308.28, and 140.7 thousand Rials per month, respectively (0.64, 0.77, and 0.35% of income). In addition, the welfare costs of tax policies (policy 4–6) for HHs were 66.78, 43.26, and 60.9 thousand Rials per month, respectively (0.16, 0.1, and 0.15% of income). There is heterogeneity in the welfare changes of policies between low- and high-income HHs. In terms of welfare changes, high-income HHs will experience a lower welfare cost due to tax policies relative to their income (0.09–0.11% vs. 0.14–0.28%). In addition, in subsidy policies, low-income HHs receive a larger welfare benefit as a share of their income as compared to high-income HHs (−0.46 to −0.88% vs. −0.28% to −0.81%; **Table 6**).

DISCUSSION

This study modeled the suggested food-related fiscal policies by experts on food demand in Iran. According to the experts, subsidy policies would have a greater impact on health, but a lower chance of stability, whereas these policies would have a higher possibility of acceptance by the society. Since subsidies impose a lot of costs on the government, it is expected that they have a lower chance of acceptance by authorities. A 2020 study by Blakely et al. raised concern that there are multiple considerations regarding fiscal policies, such as political and social acceptability and costs of any tax or subsidy (22). To the best of our knowledge, this is the first study that has been subjected to such a process to determine the most appropriate food-related fiscal policy.

We estimated a set of elasticities to simulate percent changes, nutrient intake, and welfare losses (or gains) under different policy scenarios. Our estimations are based on the all HHs, as well as HHs of first and third income tertiles, recognizing that HHs in different tertiles may have different preferences. The low-income HHs in our sample are less price responsive (except for sugar and sweets and sweetened beverages) than the high-income HHs, and so the changes in their demands were less than those of the high-income HHs. This may be because low-income

TABLE 3 | Food group statistics by household income group.

	Unit value (thousand rials)	Share (%)	Quantity (kg/liter)
All HHs			
Cereals	59.59	7.01	46.95
Un-hydrogenated vegetable oils	128.69	0.69	2.13
Hydrogenated oils and animal fats	209.99	0.66	1.27
Red meat	746.76	3.41	1.82
White meat and eggs	209.91	3.85	7.7
Dairies	83.42	2.14	10.24
Sugar and sweets	111.72	0.99	3.56
Fruits	94.28	3.32	13.95
Vegetables	48.66	2.9	23.78
Legumes	228.81	0.9	1.57
Sweetened beverages	50	0.41	3.3
Low income HHs (tertile 1)			
Cereals	46.2	9.99	52.04
Un hydrogenated vegetable oils	122.64	0.97	1.92
Hydrogenated oils and animal fats	166.74	1.03	1.5
Red meat	658.98	3.52	1.29
White meat and eggs	189	5.44	7.23
Dairies	76.86	2.86	9.05
Sugar and sweets	107.1	1.61	3.63
Fruits	78.96	3.63	11.55
Vegetables	45.36	4.17	21.98
Legumes	214.2	1.25	1.41
Sweetened beverages	48.76	0.57	2.78
High income HHs (tertile 3)			
Cereals	69.3	5.31	58.13
Un hydrogenated vegetable oils	133.56	0.5	2.87
Hydrogenated oils and animal fats	262.06	0.49	1.46
Red meat	769.44	3.21	3.19
White meat and eggs	231	2.96	10.3
Dairies	88.62	1.7	14.69
Sugar and sweets	116.34	0.67	4.42
Fruits	108.36	2.99	20.74
Vegetables	51.24	2.19	32.29
Legumes	240.24	0.68	2.16
Sweetened beverages	51.66	0.34	5.24

HHs: households. Unit values are expressed in Thousand Rials. Quantities are measured in kg or L depending on each food group. Shares represent the fraction of the total income (%).

TABLE 4 | Percentage change in household purchases due to fiscal policies.

	Policy 1	Policy 2	Policy 3	Policy 4	Policy 5	Policy 6
All HHs						
Cereals	0.92	0.46	−0.71	0.55	−0.35	0.52
Un-hydrogenated vegetable oils	−1.81	−1.06	3.5	1.58	0.97	1.56
Hydrogenated oils and animal fats	1.14	1.12	1.07	−1	−0.78	−27
Red meat	−0.46	−0.41	2.49	0.43	0.46	−2.92
White meat and eggs	2.28	−0.46	2.12	−1.51	2.34	0.62
Dairies	−0.67	−2.46	0.26	1.32	1.88	0.005
Sugar and sweets	−2.11	−2.59	−0.12	−18.7	−3.52	−2. 8
Fruits	−0.95	13.07	0.6	−0.86	1.39	−0.79
Vegetables	7.49	6.45	−1.37	−3.14	1.47	−1.2
Legumes	1.4	−3.25	30.24	−1.66	1.89	0. 8
sweetened beverages	2.75	2.87	0.42	1.18	−13.8	−2.44
Low income HHs (tertile 1)						
Cereals	1.31	0.72	−1.22	0.77	−0.48	1.19
Un hydrogenated vegetable oils	−1.03	−0.96	−0.82	−1.15	2.78	1.66
Hydrogenated oils and animal fats	2.11	1.78	4.45	2.04	−2.73	−14.69
Red meat	2.46	2.83	1.23	−0.93	2.66	−2.31
White meat and eggs	1.77	0.47	0.92	0.96	−0.97	−0.39
Dairies	−2.56	−2.54	1.8	2.12	−1.06	−0.79
Sugar and sweets	−1.74	0.83	0.18	−23.78	−1.49	0.38
Fruits	3.97	13.43	1.88	−1.96	−1.52	−2.31
Vegetables	5.5	2.81	2.97	−0.85	0.33	−1.31
Legumes	3.85	4.97	29.37	−0.33	1.53	−3.84
Sweetened beverages	0.97	1.5	−4.48	3.54	−17.76	−1.48
High income HHs (tertile 3)						
Cereals	0.1	−0.59	0.13	−0.01	0.33	0.9
Un hydrogenated vegetable oils	−0.53	1.52	1.53	2.95	−4.5	5.61
Hydrogenated oils and animal fats	2.07	2.32	2.81	2.1	−0.87	−31.87
Red meat	−1.97	0.16	−2.25	−0.44	−1.79	−2.81
White meat and eggs	0.14	0.94	−2.45	0.21	0.22	−1.51
Dairies	2.63	0.02	3.35	−1.64	−1.97	1.4
Sugar and sweets	1.83	−0.62	1.78	−21.6	−4.89	0.56
Fruits	−3.31	20.62	−0.06	−0.71	−3.22	−2.73
Vegetables	9.63	−0.47	−2.19	−0.41	0.51	0.76
Legumes	2.75	−0.01	32.47	3.22	−2.9	5.37
Sweetened beverages	1.39	1.67	−0.61	−2.03	−13.2	−2.7

*HHs: households. Each policy is defined in **Table 1**. Underlined estimates differ significantly from 0 at the 5% significance level.*

families devote a large percentage of their budget to these food categories (35.04%) rather than high-income families (21.04%). As a comparison, Caro et al. found that low-income HHs report a lower own-price elasticity for the majority of food categories except for sweets and snacks (10). Moreover, Caro et al. in another study reported that SSBs, sweets and desserts, and salty snacks are more elastic for low-income HHs when compared with high-income HHs (23).

For understanding the health implications of these policies, we converted the percent changes in HH purchases into calorie and nutrient intakes per AME per month. Consistent with the Cobiac et al's study (24), our results showed that tax policies decreased calorie intake and subsidy policies increased it. Among all the tax policies, sugar and sweets taxation was the most likely policy to lower calorie intake in the all HHs and among low- and high-income HHs. As expected, subsidy on fruits, vegetables, and legumes increased dietary fiber intake. The hydrogenated oil and animal fat tax reduced saturated fats, and the sugar and sweetened beverages tax reduced sugar intake. In addition, we found that some food taxes and subsidies could have unintended substitution effects, where, for example, a legumes subsidy might increase saturated fat consumption and limit health gains, or even cause harm. In previous simulation studies, it was shown that when food-related fiscal policies were implemented, some deleterious substitutions were expected, but the net health benefits were still high (22, 25).

In the present study, CV was used to estimate the costs of policies. As a result, the subsidy on the fruits was the most costly for the government (308.28 thousand Rials per HH as an indirect transfer to HHs). In absolute terms, we found that low-income

TABLE 5 | Change in nutrient intake per AME per month.

	Policy 1	Policy 2	Policy 3	Policy 4	Policy 5	Policy 6
All HHs						
Kilocalories (kcal)	538.48	509.21	685.03	−949.67	−144.23	−933.12
Carbohydrate (gr)	113.34	136.75	9.34	−248.42	−77.33	−5.45
Protein (gr)	30.48	13.49	43.18	−6.75	10.25	6.12
Fat (gr)	−2.18	2.47	53.84	0.48	9.75	−115.15
Saturated Fat (gr)	1.04	0.41	8.06	−1.14	1.72	−21.86
Sugar (gr)	−14.92	27.77	6.5	−235.13	−39.89	−39.94
Vitamin C (mg)	60.31	224.39	2.8	−39.45	22.05	−22.85
Dietary Fiber (gr)	11.36	15.02	16.36	−3.38	2.49	0.36
Low income HHs (tertile 1)						
Kilocalories (kcal)	935.29	1348.41	276.26	−971.68	−450.04	−245.55
Carbohydrate (gr)	177.11	287.33	−30.18	−273.04	−105.11	91.71
Protein (gr)	38.91	41.31	26.95	9.36	−8.63	4.77
Fat (gr)	13.65	13.48	32.11	−0.89	2.49	−72.06
Saturated Fat (gr)	3.36	2.86	6.5	0.71	−0.9	−14.22
Sugar (gr)	−1.1	55.68	21.13	−292.35	−29.03	−7.61
Vitamin C (mg)	89.61	165.94	49.11	−25.16	−22.98	−39.43
Dietary Fiber (gr)	14.41	18.68	16.49	0.55	−1.55	−0.9
High income HHs (tertile 3)						
Kilocalories (kcal)	665.89	795.11	959.63	−1148.03	−915. 4	−420.48
Carbohydrate (gr)	126.95	128.06	108.13	−361.96	−105.36	110.97
Protein (gr)	15.1	10.94	34.28	−17.3	−2.5	9.98
Fat (gr)	5.66	40.3	37.31	33.34	−52.31	−106.91
Saturated Fat (gr)	1.07	5.67	4.31	4.24	−6.21	−24.21
Sugar (gr)	35.28	125.7	35.23	−301.17	−92.81	−1.35
Vitamin C (mg)	54.07	375.77	−15.8	−18.6	−63.12	−39.1
Dietary Fiber (gr)	12.67	16.56	22.34	1.15	−3.25	6.3

*HHs: households. Each policy is defined in **Table 1**. Underlined estimates differ significantly from 0 at the 5% significance level.*

TABLE 6 | Households welfare change (compensated variation as % of income and Thousand Rials per month) due to price changes.

		All HHs		Low income HHs		High income HHs	
		% of income	Thousand Rials	% of income	Thousand Rials	% of income	Thousand Rials
Subsidy scenarios	20% on vegetables	−0.64	−254.52	−0.88	−214.2	−0.5	−347.76
	20% on fruits	−0.77	−308.28	−0.85	−206.64	−0.81	−561.54
	30% on legumes	−0.35	−140.7	−0.46	−111.72	−0.28	−196.14
Tax scenarios	25% on sugar and sweets	0.16	66.78	0.28	68.46	0.11	78.96
	30% on sweetened beverages	0.1	43.26	0.14	34.86	0.09	64.26
	30% on hydrogenated oil and animal fats	0.15	60.9	0.25	62.58	0.11	79.8

HHs: households. Positive values denote welfare losses, whereas negative values denote welfare gains. Underlined estimates differ significantly from 0 at the 5% significance level.

HHs experience the largest relative welfare loss and gain (as a share of income) for tax and subsidy policies, respectively. The results of the current study confirm those of Zhen et al. (26) who found that the amount of compensation to erase direct welfare loss from increasing the price of sugar-sweetened beverages is much greater for low-income HHs in the United States. Evidence from Chile also indicates tax policy will result in a lower welfare cost for high-income HHs when compared with low-income HHs, relative to their average monthly income (10). Thus, welfare changes across socioeconomic subgroups depend not only on the price elasticity of demand but also on the initial level of consumption.

This study has a number of limitations. First, As noted in other studies (10, 23), we only examined the demand for foods and beverages and did not consider other substitutions and income effects beyond these purchases. Second, the data did not allow us to further separate food items, (e.g., whole grains from other cereals). Third, we examined the food-related fiscal policy effect on purchasing patterns and nutrient intake but did not consider the health impact of the policies. Fourth, we assumed that price changes would be fully passed onto the consumers, if it was not fully (but partially) passed onto them, the welfare changes from the policies would be undermined. The main strength of our study was using AME to calculate calorie and nutrient intake, as well as FAO estimates of waste percentages in order to be close to the real consumption rates of the individuals and to avoid possible underestimation and overestimation.

CONCLUSION

As fiscal policies are increasingly gaining policy makers' attention to promote healthier purchases, our estimates provide essential information for decision makers for the implementation of such policies. Our results indicated that the demand responses to the price policies that we have studied were strongest for legumes and weakest for vegetables in all, low-income HHs, and high-income HHs. Panelists also agreed that subsidies for vegetables would have the greatest impact on health and would have the greatest likelihood of being accepted by society, as well as being the most expensive to implement. Moreover, we found that low-income HHs experience the largest relative loss and gains in welfare (as a share of income) in response to taxes and subsidies, respectively.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HE-Z: conceptualization, writing – review and editing, and supervision. MT: study design, guidance, and review of data analysis. AM-Y: writing – original draft preparation, methodology, software, and investigation. NO: conceptualization and writing – review and editing. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.917932/full#supplementary-material

REFERENCES

1. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. (2016) 388:1659–724.
2. Vettori V, Lorini C, Milani C, Bonaccorsi G. Towards the implementation of a conceptual framework of food and nutrition literacy: providing healthy eating for the population. *Int J Environ Res Public Health.* (2019) 16:5041. doi: 10.3390/ijerph16245041
3. Buyuktuncer Z, Ayaz A, Dedebayraktar D, Inan-Eroglu E, Ellahi B, Besler HT. Promoting a healthy diet in young adults: the role of nutrition labelling. *Nutrients.* (2018) 10:1335.doi: 10.3390/nu10101335
4. Mytton OT, Clarke D, Rayner M. Taxing unhealthy food and drinks to improve health. *BMJ.* (2012) 344:e2931. doi: 10.1136/bmj.e2931
5. World Health Organization [WHO]. *Fiscal Policies for Diet and Prevention of Noncommunicable Diseases: Technical Meeting report; 5–6 May 2015.* Geneva: World Health Organization (2016).
6. Epstein LH, Jankowiak N, Nederkoorn C, Raynor HA, French SA, Finkelstein E. Experimental research on the relation between food price changes and food-purchasing patterns: a targeted review. *Am J Clin Nutr.* (2012) 95:789–809. doi: 10.3945/ajcn.111.024380
7. Guerrero-López CM, Unar-Munguía M, Colchero MA. Price elasticity of the demand for soft drinks, other sugar-sweetened beverages and energy dense food in Chile. *BMC Public Health.* (2017) 17:180. doi: 10.1186/s12889-017-4098-x
8. Schwendicke F, Stolpe M. Taxing sugar-sweetened beverages: impact on overweight and obesity in Germany. *BMC Public Health.* (2017) 17:88. doi: 10.1186/s12889-016-3938-4
9. Blakely T, Nghiem N, Genc M, Mizdrak A, Cobiac L, Mhurchu CN, et al. Modelling the health impact of food taxes and subsidies with price elasticities: the case for additional scaling of food consumption using the total food expenditure elasticity. *PLoS One.* (2020) 15:e0230506. doi: 10.1371/journal.pone.0230506
10. Caro JC, Valizadeh P, Correa A, Silva A, Ng SW. Combined fiscal policies to promote healthier diets: effects on purchases and consumer welfare. *PLoS One.* (2020) 15:e0226731. doi: 10.1371/journal.pone.0226731
11. Broeks MJ, Biesbroek S, Over EA, van Gils PF, Toxopeus I, Beukers MH, et al. A social cost-benefit analysis of meat taxation and a fruit and vegetables subsidy for a healthy and sustainable food consumption in the Netherlands. *BMC Public Health.* (2020) 20:643. doi: 10.1186/s12889-020-08590-z
12. Andreoli V, Bagliani M, Corsi A, Frontuto V. Drivers of protein consumption: a cross-country analysis. *Sustainability.* (2021) 13:7399. doi: 10.3390/su13137399
13. Alagiyawanna A, Townsend N, Mytton O, Scarborough P, Roberts N, Rayner M. Studying the consumption and health outcomes of fiscal interventions (taxes and subsidies) on food and beverages in countries of different income classifications; a systematic review. *BMC Public Health.* (2015) 15:887. doi: 10.1186/s12889-015-2201-8
14. Plan and Budget Organization. *Consumer Price Index.* Available online at: https://www.amar.org.ir/english/Statistics-by-Topic/Price-indices#2225496-releases (Accessed March 11, 2022).
15. Layani G, Bakhshoodeh M, Aghabeygi M, Kurstal Y, Viaggi D. The impact of food price shocks on poverty and vulnerability of urban households in Iran. *Bio Based Appl Econ.* (2020) 9:109–25.
16. Akbari A, Ziaei MB, Ghahremanzadeh M. Welfare impacts of soaring food prices on Iranian urban households: evidence from survey data. *Int J Bus Dev Stud.* (2013) 5:23–38.
17. Ghahremanzadeh M, Ziaei MB. Food price change and its welfare impact on Iranian households. *Int J Agric Manage Dev.* (2014) 4:313–23.
18. Deaton A, Muellbauer J. An almost ideal demand system. *Am Econ Rev.* (1980) 70:312–26.
19. Tiezzi S. The welfare effects and the distributive impact of carbon taxation on Italian households. *Energy Policy.* (2005) 33:1597–612. doi: 10.1016/j.enpol.2004.01.016
20. Weisell R, Dop MC. The adult male equivalent concept and its application to household consumption and expenditures surveys (HCES). *Food Nutr Bull.* (2012) 33:157–62. doi: 10.1177/15648265120333S203
21. Gustafsson J, Cederberg C, Sonesson U, Emanuelsson A. *The Methodology of the FAO Study: Global Food Losses and Food Waste-Extent, Causes and Prevention – FAO, 2011.* Rome: SIK Institutet för livsmedel och bioteknik (2013).
22. Blakely T, Cleghorn C, Mizdrak A, Waterlander W, Nghiem N, Swinburn B, et al. The effect of food taxes and subsidies on population health and health costs: a modelling study. *Lancet Public Health.* (2020) 5:404–13. doi: 10.1016/S2468-2667(20)30116-X
23. Caro JC, Ng SW, Taillie LS, Popkin BM. Designing a tax to discourage unhealthy food and beverage purchases: the case of Chile. *Food Policy.* (2017) 71:86–100. doi: 10.1016/j.foodpol.2017.08.001
24. Cobiac LJ, Tam K, Veerman L, Blakely T. Taxes and subsidies for improving diet and population health in Australia: a cost-effectiveness modelling study. *PLoS Med.* (2017) 14:e1002232. doi: 10.1371/journal.pmed.1002232
25. Nnoaham KE, Sacks G, Rayner M, Mytton O, Gray A. Modelling income group differences in the health and economic impacts of targeted food taxes and subsidies. *Int J Epidemiol.* (2009) 38:1324–33. doi: 10.1093/ije/dyp214
26. Zhen C, Finkelstein EA, Nonnemaker JM, Karns SA, Todd JE. Predicting the effects of sugar-sweetened beverage taxes on food and beverage demand in a large demand system. *Am J Agric Econ.* (2014) 96:1–25. doi: 10.1093/ajae/aat049

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REVIEW
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The Burden of Cancer, Government Strategic Policies, and Challenges in Pakistan: A Comprehensive Review

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Cancer is a severe condition characterized by uncontrolled cell division and increasing reported mortality and diagnostic cases. In 2040, an estimated 28.4 million cancer cases are expected to happen globally. In 2020, an estimated 19.3 million new cancer cases (18.1 million excluding non-melanoma skin cancer) had been diagnosed worldwide, with around 10.0 million cancer deaths. Breast cancer cases have increased by 2.26 million, lung cancer by 2.21 million, stomach by 1.089 million, liver by 0.96 million, and colon cancer by 1.93 million. Cancer is becoming more prevalent in Pakistan, with 19 million new cancer cases recorded in 2020. Food adulteration, gutkha, paan, and nutritional deficiencies are major cancer risk factors that interplay with cancer pathogenesis in this country. Government policies and legislation, cancer treatment challenges, and prevention must be revised seriously. This review presents the current cancer epidemiology in Pakistan to better understand cancer basis. It summarizes current cancer risk factors, causes, and the strategies and policies of the country against cancer.

Keywords: cancer epidemiology, healthcare policy, health services, burden of disease, food adulteration

INTRODUCTION

Cancer is one of the arduous diseases in which the entry and spread of irregular cell development to other body regions occur. Cancer signs and symptoms depend on cancer grade and type. There are many causes of cancer in which, mostly inherited genetic abnormalities (such as BRCA1 and BRCA2 mutations) (1), infections (2), environmental factors (such as air pollution) (3), and bad lifestyle choices (such as smoking and high alcohol use) (4), may damage DNA and cause cancer. Fatigue, weight loss, skin changes, unusual bleeding, persistent cough, fever, lump, and tissue mass are the common symptoms of this disease (5). Different drug strategies have been developed to treat cancer in different conditions (6). Carcinoma affects the skin, lungs, breast, pancreas, and other organs. Sarcoma is a kind of cancer that affects the joints, muscle, fats, arteries, collagen, and other collagenous tissues of the body. Melanoma occurs in cells that make pigments in the skin (7).

Lymphoma is a cancer of lymphocytes and leukemia occurs in the blood. Breast, leukemia, lips, and oral void space cancer are the top 3 tumors in all age groups and gender (8). According to Shaukat Khanum Memorial Cancer Hospital and Research Center Lahore (SKMCH&RC) (9), the three most common cancers among young women are breast, ovary and uterine adnexa, and lip and oral hollow space cancers (9).

The three most common cancers among adult men are genital, intestine canal/anus, and lip and oral hollow space cancers (9). The most common cancers in adults, regardless of gender, are malignant neoplasms of the chest (10), lips and mouth (11), and intestine canal/anus (12). Non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) are the most frequent cancers in children (13). NHL in children may occur at any age, although it is more frequent in younger children. Adolescents are more likely to get HL (13). In children, there is no recognized cause of lymphoma. Young males with the least frequently diagnosed cancers are stomach, prostate, and mouth (14), while mature girls are commonly diagnosed with chest, cervical, and intestine canal/anus cancers (15).

The 8th edition of the American Joint Committee on Cancer staging recommendations, Tumor nodes and metastasis (TNM), classifies malignancies into four stages from 0 to 4 and unstageable and no longer acceptable tumors, according to those of unstageable tumors to all publicly available cancer websites and registry (16). According to the reports, 0.8% of the 6,291 analytical cases were assigned to stage 0, 12.9% to stage-I, and 27.2% to stage II. There were 25.4% in stage III and 18.2% in stage IV, with 10.2% getting no level and 5.3% implacable (9).

Mechanisms of Cancers

The cancer spreads as the normal processes that govern cell activity fail. A single mobile is becoming the father of many cells with unusual abilities or behaviors. It is often the result of cells accumulating genetic damage over time. Sustained proliferative signaling (17), cell death resistance (18), invasion and metastasis activation (19), and angiogenesis induction (20) are substitutes for cancer (21, 22). **Figure 1** shows how most cells inside an individual are vulnerable to DNA damage. An individual's cells usually grow and divide in a highly controlled way known as a mobile cycle throughout their lives (23). It helps tissues to mature but also live a healthy life. Until a mobile phase cell divides, it must replicate its DNA (and hence its genetic code) so that each daughter cell has DNA equivalent to the parent cell. DNA replication is a complicated process prone to sequencing errors (24).

Cells are constantly found to have elements that could harm DNA (25). Exogenous factors, such as rays or chemicals in cigarette smoke, and endogenous indicators, such as free radicals or toxic metabolites, are present in the body (26). A carcinogen is a chemical or agent capable of producing cancer, even if not all cancer-causing substances destroy DNA immediately (27). Endogenous defensive mechanisms are less effective due to inherent genetic abnormalities, excessive amounts of exposure to external cancer-causing chemicals, and endogenous elements that damage DNA integrity (27). Unsuitable nutrients over the whole basis represent a disordered dietary microenvironment at the cell level. This can lead to an environment that encourages the buildup of DNA damage and, as a result, the development of cancer stated to the World Cancer Research Fund (WCRF) (22).

GLOBAL BURDEN OF CANCER

Cancer is the leading killer, with more than 10 million deaths in 2020 (28, 29). According to the cancer statistics 2020 (29), breast cancer (2.26 million cases) (29), lung cancer (2.21 million cases) (29), intestine and rectum cancer (1.93 million cases), prostate cancer (1.93 million cases), carcinoma (1.20 million cases), and stomach cancer (1.20 million cases) are the most common cancers in the United States in 2020 (in terms of new cases; 1.09 million cases) (29). The shortage of oxygen in the body is the leading cause of cancer mortality. Breast cancer has affected almost 2.3 million people worldwide in 2020, including 0.685 million fatalities (30). The new cases and deaths are presented in **Table 1**.

Cancer Situation in Pakistan

International Agency for Research on Cancer (IARC) has reported in Pakistan that the proportion of newly diagnosed cancers is 0.18 million, the number of cancer fatalities is 0.11 million, and the number of prevalent cases (5 year) is 0.32 million (31). In Asia, Pakistan regionally represents the most significant breast cancer rate (32). Breast cancer has grown increasingly frequent in Pakistan, with one out of nine women now having a lifetime risk of the disease (33). Pakistan has one of the highest breast cancer mortality rates globally (34). Lips and mouth cancer is the 2nd most frequent malignancy in Pakistan and the top among males when both genders are included (15.9%) (35). The increased usage of smokeless tobacco, such as areca nut, can increase the burden of severe lung tumors (SLT) (36). SLT is a collection of over 30 low and high-toxic substances (37). The list of cancers, number of deaths, and new cases in Pakistan in 2020 are presented in **Table 2**.

Abbreviations: Brca1, breast cancer gene-1; Brca2, breast cancer gene-2; SKMCH&RC, Shaukat Khanum Memorial Cancer Hospital and Research Center; NHL, Non-Hodgkin lymphoma; HL, Hodgkin lymphoma; TNM, tumor nodes and metastasis; WCRF, World Cancer Research Fund; SLT, severe lung tumors; IDA, International Development Association; PNNSKR, Pakistan National Nutritional Survey Key Report; NCR, National Cancer Registry; NPFPPH, National Program for Family Planning and Primary Health Care; PAEC, Pakistan Atomic Energy Commission; HRDP, Human Resource Development Program; CCHRC, Cancer care hospital and research Centre Pakistan; CCFPT, Children Cancer Foundation Pakistan Trust; CCH, Children Cancer Hospital, Pakistan; NCDBP, National Cancer Data Base Pakistan; PSCO, Pakistan Society of Clinical Oncology; NHSRC, National Health Services Regulations and Coordination; PHRC, Pakistan Health Research Council; HRCP, Human Rights Commission Pakistan; PCCWS, Pakistan Cancer Care Welfare Society; PIMS, Pakistan Institute of Medical Sciences; SOS-PK, Surgical Oncology Society of Pakistan; NAP-NCD, National Action Plan for Non-Communicable Disease Prevention, Control, and Health Promotion in Pakistan; NCD, non-communicable diseases; SROs, statutory regulatory orders; NMSC, non-melanoma skin cancer; GLOBACAN, global cancer incidence, mortality and prevalence; IARC, International agency for research on cancer; AICR, American Institute of cancer research; PG, pyogenic granuloma; TNF, tumor necrosis factor; CYP, cytochrome P450; GST, glutathione S-transferase; WRA, women of reproductive age.

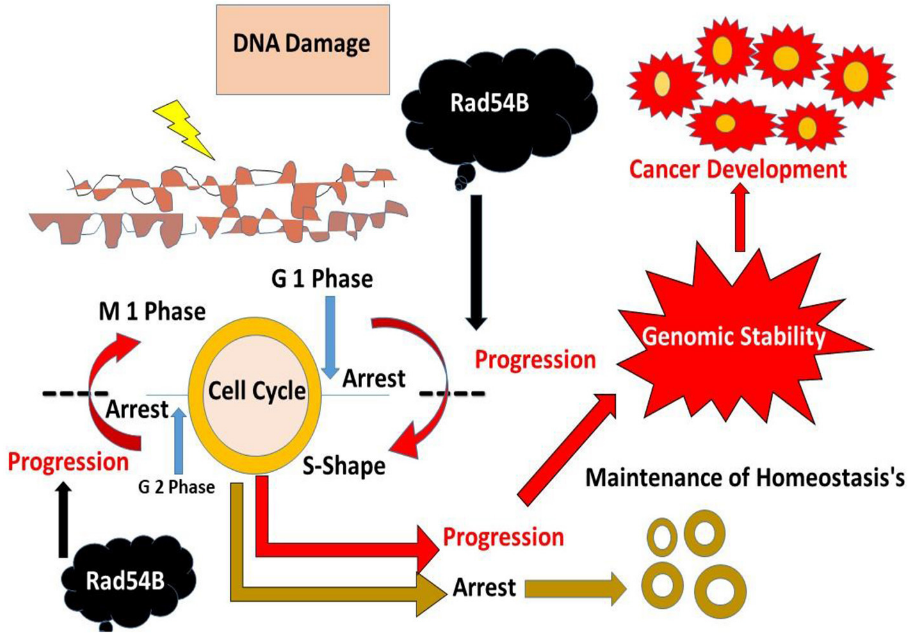

FIGURE 1 | The different mechanisms of cancer and its progression.

TABLE 1 | New cases and deaths for different types of cancer worldwide. (Data have been acquired from Global Cancer Statistics 2020).

Cancer type	New cases (% of all sites)		Death (% of all sites)	
	Number	%age	Number	%age
Gastrointestinal	3,573,928	18.5	2,228,749	22.4
Leukemia	474,519	2.5	311,594	3.1
Melanoma of skin	1,198,073	6.2	63,731	0.6
Brain/Nervous system	308,102	1.0	99,840	1.0
Pulmonary cancer	2,707,406	14.7	2,019,937	20.3
Genitourinary	4,017,064	21	1,548,189	15.6
Liver cancer	1,401,450	7.3	1,296,183	13
Mouth/Oral cavity cancer	431,296	2.3	167,235	2.0
Breast cancer	2,261,419	11.7	684,996	6.9
Multiple myeloma	176,404	0.9	117,077	1.2
Mesothelioma	30,870	0.2	26,278	0.3
Hodgkin lymphoma (HL)	83,087	0.4	23,376	0.2
Mesothelioma	30,870	0.2	26,278	0.3
Kaposi sarcoma	34,270	0.2	15,086	0.2
Others	2,564,031		1,329,584	
Sum of all sites	19,292,789		9,958,133	

TABLE 2 | New cancer cases and death for different types of cancer in Pakistan (Data have been acquired from Global Cancer Statistics 2020).

Cancer type	New cases (% of all sites)		Death (% of all sites)	
	Number	%age	Number	%age
Gastrointestinal	23,220	14.87	21,077	18.08
Leukemia	8,305	4.7l	6,261	5.3
Melanoma of skin	502	0.28	290	0.25
Brain/Nervous system	4,770	2.7	3,934	3.4
Pulmonary tract cancer	19,008	10.61	14,488	12.31
Genitourinary	25,241	11.35	11,026	9.46
Liver Cancer and Gallbladder	8,372	4.7	7,739	6.6
Mouth/Oral cavity cancer	20,620	10.01	11,761	10.07
Breast cancer	25,928	14.5	13,725	11.7
Multiple myeloma	1,978	1.1	1,726	1.5
Mesothelioma	41	0.02	34	0.03
Non and Hodgkin lymphoma (NHL)	8,305	4.63	4,550	4.36
Mesothelioma	41	0.02	34	0.03
Kaposi sarcoma	77	0.04	51	0.04
Others	31,980	–	20,453	
Sum of all sites	178,388	–	117,149	–

POTENTIAL RISK FACTORS OF CANCER IN PAKISTAN

Diet and Nutrition

Malnutrition was common among advanced cancer patients (38). The nutritional deficiency was linked to poor clinical outcomes (38). According to numerous studies, poor nutrition is prevalent among cancer patients, with rates ranging from 31 to 97% (39–42). Malnutrition can result in impaired immunity (43), higher infection rates (44), inadequate reaction and endurance for therapy (45), higher healthcare costs (46), worse quality of life (47), and shorter survival durations (48).

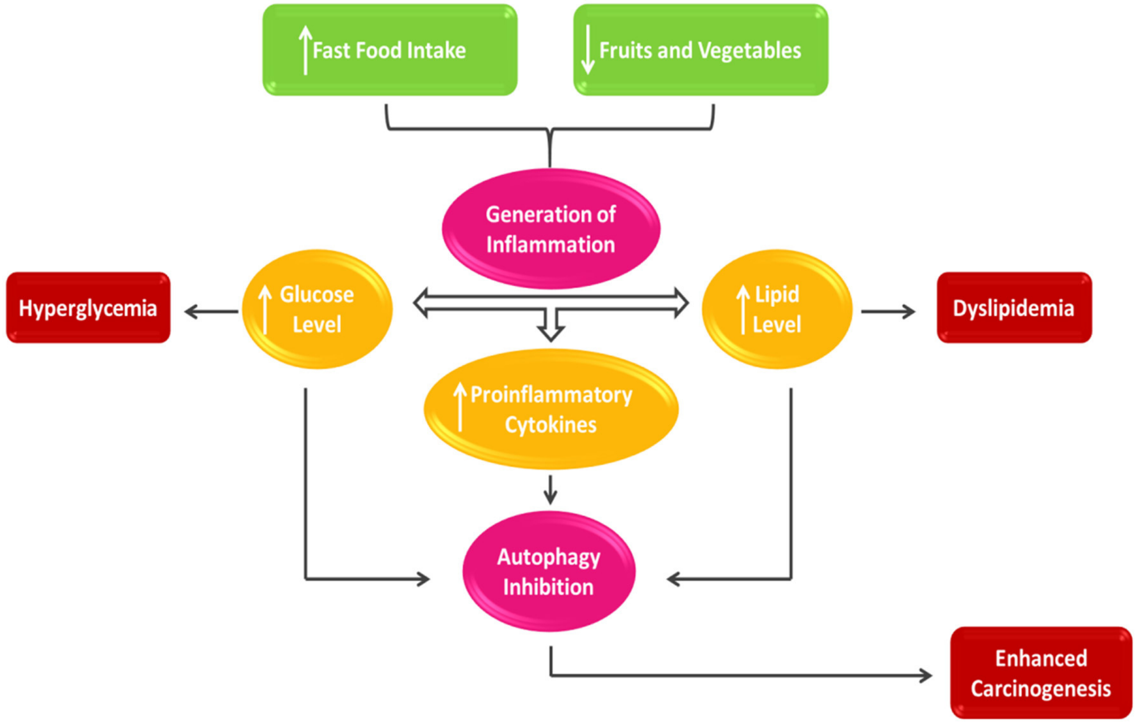

FIGURE 2 | Nutrient intake and cancer prognosis.

Dietary Consumption

Cereals continue to be the mainstay of the Pakistani diet, accounting for 62% of total energy (49). Pakistan has a high level of dietary patterns compared to other Asian countries (50). However, there is a lack of diet quality and fish and meat consumption (51). Fruit and fresh vegetables, which are sensitive to local seasonal availability, are similarly limited due to the country's lack of established marketing facilities. Micronutrient deficiency disorders in Pakistan are likely to be caused by fluctuations in the availability of these essential foods (52). **Figure 2** shows the link between dietary consumption and cancer.

A healthy diet rich in fruits and vegetables has been related to a decreased risk of cancer. Consumption of vegetables reduces cancer risk, according to American Institute of Cancer Research (AICR) (53). As a result, it is assumed that vegetables contain chemopreventive chemicals. Isothiocyanates and myo-inositol have been the emphasis. Isothiocyanates, found as glucosinolates in green vegetables like mustard, cabbage, lettuce, radish, and cauliflower, seem plentiful (54). Beans, cereals, and nuts all contain myo-inositol (55). These compounds inhibited the enzymatic activity of 4-(N-Methyl-N-nitrosamine)-1-(3-pyridyl)-1-butanone (56, 57).

Epidemiological and experimental studies indicate that Brussels sprouts, kale, broccoli, and cabbages have anticancer properties (58). Sulforaphane, a phytochemical found in cruciferous vegetables such as broccoli, Brussels sprouts, sorghum and cabbage, has anticancer characteristics (58, 59). Cabbage has antioxidant and anti-inflammatory properties that could help prevent cancer (60). Foods that increase the bioavailable content of non-heme iron and alternative treatments for cancer patients can all benefit from fresh cabbage juice, whether made individually or combined with other vegetables like carrot and celery (61, 62) Thiocynate obtained from the hydrolysis of *Brassica oleraceae* has anticancer and antioxidant properties (60, 63). Cauliflower and cruciform vegetable intake has reduced cancer rates (64). Radish sprouts may have a more substantial chemoprotective effect against carcinogens than broccoli sprouts (65). Antimutagenic efficacy of aqueous extract of salted radish roots against *Salmonella typhimurium* TA98 and TA100 (66). Radish sprouts have a high concentration of glucoraphanin, glucosinolates that hydrolyze to produce sulforaphane, a potent inducer of phase 2 detoxification enzymes with anticancer properties (65). Due to the indoles present in turnip, it has anti-tumorigenic properties (67). Substantial antimutagenic factors and hydroxyl radical scavengers have been found in Turnip seeds (68).

Flavonoids, also present in tomatoes, have been shown to prevent carcinogenesis *in vitro*, and there is strong evidence that they can do so *in vivo* (69, 70). In considerable amounts, Tomato leaf extract includes isolated active components with anticancer activity (70). Cardioprotection, anti-inflammatory, Antimutagenic, and anti-carcinogenic qualities are only a few of the biological advantages of tomato lycopene (71). Lycopene-rich tomatoes and tomato products have decreased the risk of chronic diseases such as cancer and heart disease (71). Cancer might be

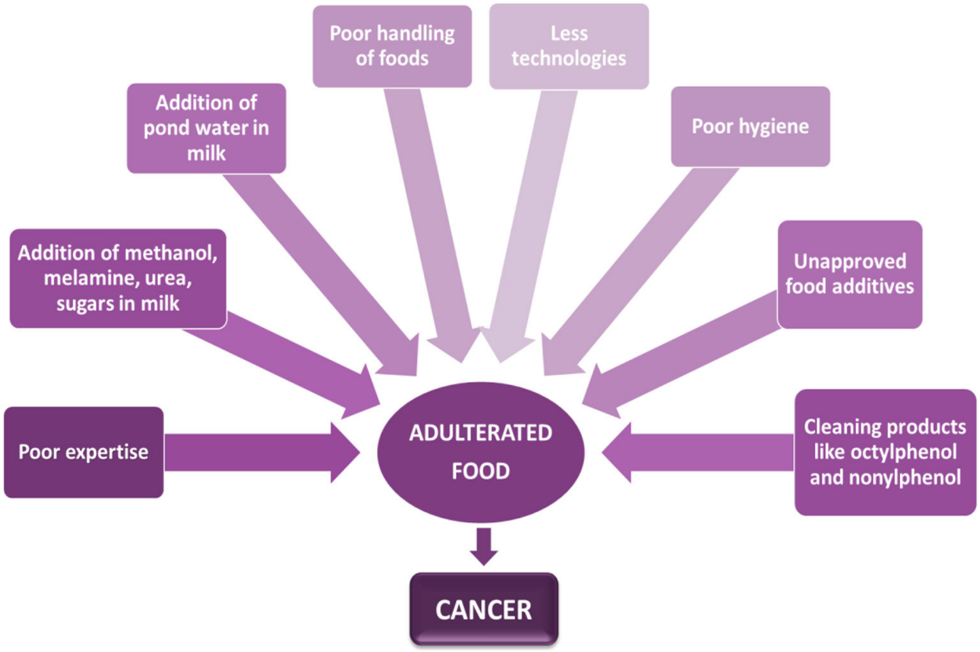

FIGURE 3 | Association between contaminated food and cancer.

prevented or delayed by delaying or inhibiting the steps leading to genetic damage or activating preventive systems (72). The large-scale (α-Tocopherol and β-carotene) cancer prevention studies and carotene and retinol effectiveness trial intervention studies found that anyone who gained early plasma or potency layers of carotenoid had a lower risk of developing lung cancer (73, 74). Those with greater pre-intervention carotene concentrations in their plasma or diets reduced cancer incidence (75). A high-carotene diet may also benefit cancer treatment (76, 77).

Adulteration

Pakistan is indeed an agricultural land, and farmland has always significantly influenced the nation's economy with cattle (78). Pakistan produced 42.199 million tons of milk last year, with buffaloes accounting for 62.17% of total milk output, cows 34.21%, sheep, goats, and camels 3.60%. Pakistan's milk production and delivery infrastructure are antiquated and insufficient, despite the country's favorable position among dairy-producing countries (79). The irregular private sectors, which are made up of many agents such as sellers, collectors, mediators, processors, merchants, and dairy stores, perform a specific function at a different stage of the production process and manage it (80). Pakistan's dairy industry is beset by many issues, including a scarcity of industrial milk production, milk expertise, and legal and technological resources (81).

Moreover, its lack of decent inspection is the system's biggest overlooked flaw. At practically every stage of the marketing process, testing is just about non-existent (82). On the other hand, rising inflation and poverty levels have made most Pakistani consumers budget-conscious. As a result, open raw milk has a higher demand than pasteurized milk (83). Different factors are shown in **Figure 3**. Water is perhaps the most basic and transparent adulteration in milk and is applied to boost the volume of a valuable product. Still, dirty water puts people's health in danger from waterborne illnesses (84).

Owing to decreased milk output, milk yield in Pakistan is reduced by 55% throughout the summer (85). However, when milk production is abundant in contrast to the spring, price increases of up to 60% are usual. Water is blended into the whole dairy throughout the summer to enhance the milk available to meet demand (86, 87). Because pond water is an excellent nitrogen resource, some unscrupulous people add it to milk to increase the relative density. Human ingestion of these water-spoiled milk can induce gastrointestinal problems, mainly in the elderly, or pose significant health risks to children and infants who regularly eat dairy (88). **Table 3** shows the many cancers induced by various foodstuffs.

TABLE 3 | Linkage of certain foods to various malignancies.

Food items	Type of cancer	References
High intake of red meat	Colorectal cancer	(89)
High salted vegetables/food	Gastric cancer	(90)
Ultra-processed food and drinks	Chronic lymphocytic leukemia	(91)
High fat	Breast cancer	(92)
Low vitamin C, E rich foods	Skin cancer	(93)
High alcoholic beverages intake	Lung cancer	(94)
High carbs	Brain tumor	(95)
High fat	Liver cancer	(96)

To avoid milk from spoiling during delivery, especially in hot weather, it is refrigerated (the ice used may be contaminated) (97). Milk is also important because it has whey protein with many health benefits (98). Methanol, melamine, urea, and sugars can be illegally polluted in milk (97). In summers, H2O2 protects milk whenever the heat is exceptionally high. Hydrogen peroxide damage to stomach cells can cause gastritis, bowel inflammation, and severe diarrhea (99). Cleaning products used to clean milk also cause cancer (86).

Fast Food and Junk Intake

Fast food is food that can be prepared and served quickly (100). They're known for offering rich, delectable food at affordable costs. Nonetheless, many people nowadays, especially the young, like fast food like burgers, chicken wings, pizza, and shawarma (101). Fast food's success can be attributed to a variety of things. The changing level of living is among the essential variables. Most individuals spend extra shifts and even whole study hours to make finances meet. They won't have time to shop for supplies or prepare delectable meals. The increasing number of young, wealthy people is one factor. Most people in most countries spend a lot of money on fast food since they are primarily young (102). Children are regularly exposed to fast food advertisements on television and the Internet (103). Youngsters enjoy dishes with vivid shades and miniature gadgets, although full of fat, salty, or artificial sweeteners. Fast-food consumption has increased dramatically in Pakistan (104). Client meal preferences are impacted by several factors, including client desire to dine out (105), networking (106), globalization (107), a need for university undergraduates (108), the convenience of Pakistani households with two incomes, and a variety of other factors (109). People are eating in a new way in terms of global with the rising relative importance of snacks, burgers, pizzas, and fizzy beverages (110). As previously stated, the need for food is linked to urban culture, and development is one of the factors contributing to changing lifestyles, increasing wealth, and the independence of young people (111).

Many individuals like eating fast food regularly, even though they may be unaware of its negative health impacts. One of the ailments caused by consuming fast food is cancer (112)People in Pakistan often buy and eat fast food between 6 and 9 p.m., as per findings of a study (113). Due to its delectability, fast is consumed by single and joint households in Pakistan. The nuclear family unit is more cost-conscious than the common family unit. Fast food is popular among the general public, and many people choose to dine outdoors in their houses (114). People who were overweight were more likely to eat fast food at home (115).

As a consequence, dining out is a better choice (116). There is conflicting evidence of a relationship between food quality and prostate cancer (117). According to recent research, taking in more food, fruits, and vegetables is linked to a decreased incidence of prostate cancer (118). The low-fat, high-vegetable, and fruit-intake diet can help to reduce the risk of prostate cancer (119). In several studies, a high-fat diet, particularly red meats and dairy products, has increased cancer risk (120). In South Asia, vitamin D insufficiency is relatively frequent. According to studies, 70–97% of Pakistan's primary population is deficient in vitamin D, with vitamin D insufficiency being more prevalent in cities (121). Vitamin D deficiency is widespread among Pakistan's general population (122). Vitamin D insufficiency was detected in 95% of women with breast cancer and 77% of healthy people in research from a prestigious cancer clinic in Pakistan (123). On the other hand, Chlebowski discovered no link between vitamin D deficiencies and cancer (62, 123). In earlier studies, vitamin D levels over 50 ng/ml have been linked to a 50% decreased risk of breast cancer (124, 125).

Gutkha and Paan

Tobacco use seems to have a long history in many regions, particularly India, Pakistan, other Asian nations, and America (126). There are around 28 carcinogenic chemical constituents in smokeless tobacco, the most common of which is nitrosamine (127). Due to a significant lack of information and awareness, most people utilize Paan and Gutkha (128).

Children think of Gutkha as candy because of its pleasant flavor (36). Gutkha is widely assumed to be a mouthwash (36). However, its pleasant taste and smooth texture attract bacteria, leading to tooth disease (36). In most places where Paan and Gutkha are widely used, it is difficult to control their use, and their widespread use contributes to oral cancer. Tobacco, seed, soaked lime, herbs, and geek wrapped in flake are common ingredients in paan (129). Mouth Sub Mucous Carcinoma seems to be a chronic oral condition marked by mucus buildup in the mouth, pain, and necrosis of subcutaneous soft tissue. Oral cancer is relatively frequent in Pakistan, India, China, Taiwan, Sri Lanka, Malaysia, and Indonesia (130). Gutkha intake was already proven even to have carcinogenic and chromosomal effects. In certain circumstances, drinking rather than smoking significantly impacts oral cancer (131). In Pakistan, males and females had prevalence estimates of 21.3 and 19.3%, respectively. Pakistan is the second most common country where smokeless tobacco is used (132). Tobacco products have been connected to almost 90% of mouth cancer cases, which is critical in cancer formation (133). People who inhaled smoke more than 10 times per day have been found to have a higher chance of acquiring cancer than those who did not (134). Paan is consumed by 3.3–37.0% in Pakistan and India (36). Mouth cancer is the third least frequent cancer in Pakistan and India (135), behind breast and lung cancer. Breast cancer affects more females than males (136). The use of such items is considered normal in the culture. Despite their deliciousness, Gutkha, Chaalia, paan, toombak, and naswar lead to mouth cancer (36). Numerous studies show that these goods are consumed by 20–30% of individuals and teenagers in Pakistan, India, and Nepal (137, 138). In Karachi, Pakistan, 40% of the populace chews betel nut, areca nut, and tobacco products (139). According to a study, more than 74% of pupils in Karachi, Pakistan, consume digestible items regularly (140). Paan, chaalia, gutkha, naswar, and toombak were utilized 34.3, 34.7, 46.0, and 50% of the time in Sindh, Punjab, respectively Pathan, and Mohajir districts, according to a 2006 report (141). According to a study conducted a decade ago, gutkha was consumed on a regular basis by 46% of Karachi residents (142). Another study

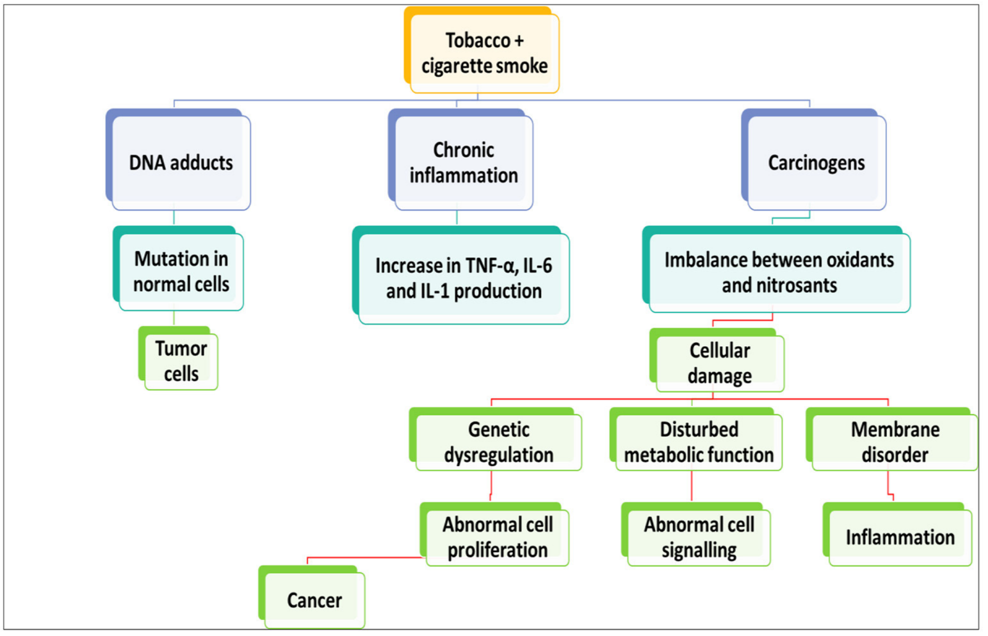

FIGURE 4 | Links between the oxidative and nitrosative induced damage and progression of cancer.

found that 35% of persons attending a medical care facility from Karachi, Pakistan, consumed paan, gutkha, or other oral tobacco frequently (143). The paan and gutkha processes have been proposed for humans, as shown in **Figure 4**.

Before swallowing, paan, gutkha, and zarda are crushed, licked, or rubbed between gums and teeth. According to the World Health Organization, Nicotine mycotoxins constitute 76–91% of N-nitroso compounds (144). Paan and gutkha produce irritation of the oral mucosa, which stimulates T-cells and macrophages, resulting in the generation of prostaglandins. The pyogenic granuloma (PG) generation by buccal cavity keratinocytes is stimulated by areca nut extract, which is critical for tissue stiffness and malignancy. Tumor necrosis factor (TNF), interleukin-6, and growth factor-like have all been produced in areas of aggravation (36).

The nitrosamine in tobacco is biologically changed *via* cyp450 enzymes, which can form N-nitrosonornicotine, a carcinogenic solid that can cause DNA damage and, as a result, oral cancer (145). cytochrome P450 (CYP), Glutathione S-transferase (GST), and TNF represent cytochrome polymorphism, glutathione-s-transferase, and cancer necrosis signals.

Reduced salivation and mucus production have been observed in paan and gutkha eaters, decreasing the oral mucosa's normal microbiota and an increased risk of infection from Aspergillus (146). In laboratory animals, Paan and gutkha have been shown to cause stomach, mouth, throat, and laryngeal cancers. To mice, paan is just a melanoma agent (147). According to studies, mice given smokeless tobacco developed malignancies in their reproductive organs, ovaries, stomach, kidneys, abdomen, and lungs. As a result, smokeless tobacco is carcinogenic to the mouth and other bodily systems (148).

Micro-Nutrients Deficiency

A deficiency of vitamin A may cause metaplastic changes in the nasal passage (149). Both inheritance and the effects of harmful practices such as smoking and eating a diet lacking plant-based items increase the chance of secondary cancer cells in many body regions (150). The relation between micro-nutrient deficiencies with cancers has been supplemented in **Table 4**.

Vitamin C insufficiency is common among south Asians and one of the contributing causes (165). Vitamin C levels in the blood of Indians and Pakistanis living in India and Pakistan have dropped dramatically (165). The primary cause might be a lack of vitamin C rich fruits and vegetables, especially among individuals from poor socioeconomic categories (166). Patients with advanced disease receiving chemo or immunotherapy may be at a higher risk of Vitamin C depletion due to increasing demands rather than a lack of consumption (167). Women had more significant amounts of ascorbate than males, while active patients had more meaningful levels than sedentary people (168). Anemia is more common in people who have had a tumor recurrence and are in the later stage of the disease. That is, from 40% of individuals with early-stage colon cancer to almost 80% of patients with advanced cancer (169). An iron shortage can be fatal (usually related to bleeding), for those with low iron reserves or who are iron deficient somehow (170). Poverty, hunger, illiteracy, inadequate infrastructure, and a lack of policy and law are all factors that contribute to the international development association (IDA) in Pakistan, according to several indicators (171).

According to the Pakistan National Nutritional Survey Key Report (PNNSKR), 2019, Vitamin D deficiency among children under five is a severe problem in Pakistan (172). Vitamin D

TABLE 4 | Micro-nutrient deficits linked with a variety of cancers.

Type of cancer	Micro-nutrients	Outcome	References
Breast cancer	Vitamin D3	In breast cancer patients, vitamin D insufficiency is a typical occurrence.	(151)
	Selenium	In 2014, a meta-analysis identified a link between selenium blood levels and the risk of breast cancer.	(152)
	Folate, zinc, beta-carotene	In a 2014 investigation, many genetic abnormalities and/or deficits in folate, zinc, and beta-carotene were linked to triple-negative breast cancer development, especially when they were identified together.	(153)
	Iodine	Iodine is a mineral found in the thyroid and breast tissue that aids in preventing breast cancer. Low iodine levels may be considered a risk factor for breast cancer due to the high prevalence of hypothyroidism in breast cancer patients.	(154)
Prostate cancer	Zinc	A study of Nigerian prostate cancer patients identified a relationship between zinc deficit and prostate cancer and selenium and vitamin E deficiency.	(155)
	Vitamin E and trace minerals	As previously indicated, a study on Nigerian males with prostate cancer was undertaken. According to this study, prostate cancer patients exhibited significantly decreased levels of whole blood superoxide dismutase (SOD), vitamin E, serum selenium, and zinc. AS A RESULT, Vitamin E, zinc, and selenium deficiency may be risk factors for prostate cancer.	(155)
	Selenium	Increased plasma/serum selenium levels (170 ng/mL) were found to lessen the incidence of prostate cancer in a comprehensive review and meta-analysis of selenium and prostate cancer.	(156)
	Vitamin D3	Vitamin D3 25(OH) D concentrations were inversely correlated with prostate cancer risk but not vitamin D–related polymorphisms or parathyroid hormone. This suggests a relationship between low vitamin D3 blood pathology and a higher risk of prostate cancer.	(157)
Colon cancer	Folic acid	In colorectal cancer treatment, folic acid is a contentious vitamin. Even though high folate levels have been linked to a lower risk of colorectal cancer, too much folate can stimulate cancer growth.	(158)
	Selenium	In animal studies, selenium deficiency has been shown to aggravate colitis and speed tumor formation and progression in inflammatory carcinogenesis.	(159)
	Vitamin D	Many colorectal cancer patients have vitamin D3 insufficiency and deficiency.	(160)
	Fiber, low fruit and vegetable, high red and processed meat intake	Even if not a single nutrient, it has long been known that a diet poor in fruits and vegetables, fiber, and red and processed meat intake is related to the development of colorectal cancer.	(161)
Lung cancer	Selenium	Several epidemiological studies have found that persons with low selenium levels in their blood had a higher risk of lung cancer, albeit the findings are contradictory. Research done in the southeast United States showed that lower-income and black Americans were more likely to get lung cancer.	(162)
	Vitamin A	Cigarette smoking has been linked to the development of lung cancer. Cigarette smoking has been shown to lower retinoic acid levels in the lungs of rats and increase the growth of precancerous and cancerous tumors.	(163)
	Vitamin D3	Vitamin D3 deficiency is common in lung cancer patients, ranging from mild to severe.	(160)
	Zinc	Human investigations on zinc deficiency and lung cancer are few and far between. Zinc deficiency has been demonstrated to cause DNA instability and undermine its integrity in cell culture studies on human lung fibroblasts, suggesting that it may have a role in preventing DNA damage and cancer.	(164)

insufficiency was found in a substantial percentage of people (62.7%) (173). Many youngsters (13.2%) suffer from a severe deficit. The incidence is somewhat higher (63.1%) among girls than it is among boys (62.4%) (174). Vitamin D insufficiency affects the majority of women of reproductive age (WRA) (79.7%), with 54.0% having a moderate deficit and 25.7% having severe deficiency (175).Vitamin D insufficiency is more prevalent in urban areas (83.6%) than in rural areas (77.1%) (175, 176). Vitamin A deficiency in a child under the age of five affects 51.5% of children, with 12.1% suffering from severe inadequacy (175, 177). Anemia is more common in those with a tumor recurrence at a later stage of their disease (from 40% of patients with early-stage colon cancers to almost 80% of patients with advanced disease) (175, 178). Iron deficiency can be absolute (typically caused by bleeding) or functional (caused by insufficient iron reserves) (175, 179). The prevalence of the disease is somewhat higher (51.7%) in males than in girls (51.3%) (174, 175). Adolescent females in rural regions are more likely than their urban counterparts to be anemic (175, 180). Zinc Deficiency in Children under the age of five zinc deficiency affects 18.6% of the population, with comparable rates in males and girls (175). Children in rural regions had a somewhat greater likelihood (19.5%) than in large cities (17.1%) (175, 181). Some cancer types related to different nutrients are supplemented in **Table 4**.

CHALLENGES

No National Cancer Registry (NCR) in Pakistan can give the actual magnitude of the problem to create a health policy (182). Pakistan has a scarcity of nationally representative statistics. There are no long-term cancer patient data available. No comprehensive cancer control plan exists (182–184). There are currently no systematic cancer prevention and control health education efforts (183). The only national initiative was the

National Program for Family Planning and Primary Health Care Pakistan (NPFPPH), which created 100 television advertisements to promote awareness of cancer's early warning signals (185). This, however, is not a long-term activity. In a few institutions, government agencies and activist groups have produced patient education materials (186); nevertheless, these activities have had limited impact. At the national, provincial, and municipal levels, a network of organizations is lacking. Low-cost public health methods to promote palliative care are lacking, particularly in low-resource areas. Due to a shortage of government money, planning was halted. Policymakers, the health sector, and other government institutions engaged in cancer care and treatment are less enthused. Pakistan lacks adequate policies and professionals for medical nutrition therapy (187). Vertical service delivery arrangements and poor performance accountability within the government are causing less efficiency and quality concerns in the health system. The private sector also duplicates services, primarily unregulated for quality treatment and cost. Pakistan's present health infrastructure are scattered and unidirectional (188). Current healthcare technology has not progressed (189). The current systems for determining the suitability of supplies, diagnostics, medications, and laboratory reagents are not based on evidence (189). There are concerns with medicine quality, pricing, and recommendations (189). The price of medicines is controversial between regulators and the pharmaceutical industry (190).

PROGRAMS AND POLICIES IN PAKISTAN

Cancer Treatment Program in Pakistan

The Pakistan Atomic Energy Commission (PAEC) has played an essential role in the health sector (191). PAEC now offers over 13 Nuclear Medicine and Oncology Centers equipped with Health and Nutrition having excellent facilities and offers to continue integrated programs to identify various malignancies and associated ailments (192). The primary disciplines accessible and in use in PAEC nuclear medical institutes are nuclear medicine, clinical oncology, surgical oncology, clinical labs, radiology, medical physics, and bioengineering (193). In addition to directing the operations of essential disciplines at several PAEC nuclear medical facilities, the Directorate General of Medical Sciences, PAEC Headquarter, is working on a Human Resource Development Program (HRDP). This would ensure that qualified and competent people in every aspect of cancer diagnosis and treatment are available (193).

Cancer Care Hospital and Research Centre (CCHRC) Pakistan

Its purpose is to provide high-quality, all-inclusive cancer treatment at no cost while addressing their physical, social, economic, and spiritual requirements (194). They also don't turn away any patients, accepting around 20,000 each year. This hospital was created due to a lack of chemotherapy facilities in Punjab 26/110, Baluchistan 1/12, Sindh 21/4, and KPK 14/35 (195). Chemotherapy takes 4–6 months, just like it did at SKMCH&RC.

Children Cancer Foundation Pakistan Trust (CCFPT)

This is intended for children only. This trust's mission is to offer cancer screening and treatment to all children, regardless of their financial situation. CCFPT was established years back with a strong direction and commitment to building a Children's Cancer Hospital (CCH) in Pakistan. Every child may receive quality cancer treatment and raise public awareness about childhood cancer (196).

National Cancer Data Base Pakistan (NCDBP)

In 2010, the NCDBP was established. The Pakistan Society of Clinical Oncology (PSCO) and its associated centers also work together (197). A cancer registry must be established to prevent and manage cancer. We now need an NCR that can offer information on the actual scope of the problem to establish health policy. The Ministry of National Health Services Regulations and Coordination (NHSRC) has tasked the Pakistan Health Research Council (PHRC) with establishing a cancer registry by affiliating all of the country's leading public and private institutions. Eight hospitals (Jinnah Postgraduate Medical Center, Karachi, Civil Hospital, Karachi, National Institute of Child Health, Karachi, Nishtar Hospital, Multan, Allied Hospital Faisalabad, Bolan Hospital, Quetta, Khyber Teaching Hospital, Peshawar, and Armed Forces Institute of Pathology, Rawalpindi) have been sending data to the Human Rights Commission Pakistan (HRCP) every quarter since May 2015 (198).

Pakistan Cancer Care Welfare Society (PCCWS)

This society's main objective is to provide financial assistance to the patient because it's challenging for someone diagnosed with cancer to manage expenses. However, Government assistance is needed too. Its mission is to help cancer patients medically, physically, socially, economically, and psychologically.

The PCCWS is a non-profit public welfare organization based in Pakistan to promote cancer awareness. PCCWS started in 2006 and presently has over 200 members and is currently striving to improve cancer awareness in lower Punjab (199). PCCWS follows the American Society of Cancer's monthly theme-based calendar system and organizes lectures, seminars, presentations, and campaigns throughout the lower Punjab, both rural and urban. PCCWS makes cancer literature in Urdu accessible, comprehensive, and up-to-date for local populations (199).

Pakistan Institute of Medical Sciences (PIMS)

The department houses Pakistan's World Health Organization (WHO) National Cancer Control Project. The Pakistan Institute of Medical Sciences treats patients from Islamabad, Khyber Pakhtunkhwa, FATA, Gilgit-Baltistan, Kashmir, and Punjab (200). In addition, PIMS provides round-the-clock service to Parliamentarians, government officials, and judicial employees (32). All components have very visible and unambiguous signposting for services provided at PIMS to assist patients and their attendants (200). All cancers are treated at the oncology department with curative purpose chemotherapy. PIMS only have eight beds in its indoor section. Despite this, it regularly has more than 14 patients since it is the only government-run clinic in the region. The daily outpatient clinic sees 40 patients every day. Outpatient chemotherapy for 10–15 patients every day (200).

With the help of Saylani Welfare Trust and the EHSAAS Program, the patient's attendants are provided with free meals three times a day (201). In the "Shelter Home," patient's attendants are also given a place to sleep at night (201). A "Sarai" is also available to accommodate the parents of pediatric patients (201).

Pakistan Society of Clinical Oncology (PSCO)

PSCO is a professional organization in Pakistan for cancer/oncology experts who have come together to fight cancer nationally (202). PSCO is the largest organization in the country, representing all of the experts who treat cancer patients (202). The society's mission is to promote and support the field of clinical oncology and associated sciences in cancer treatment (202). PSCO improves clinical oncology practice as a best-cost-effective cancer strategic approach in resource-constrained countries. PSCO encourages patients and the general public to learn more about cancer care by supporting prevention, screening programs, and accurate disease information. PSCO maintains relationships with other oncology societies, cancer forums, universities, patient groups, radiotherapy machine vendors, and the pharmaceutical business (202). Its main objectives are to assist and enhance the speciality of clinical oncology and related sciences engaged in cancer therapy and to serve the community using its diagnostic, therapeutic, and research capabilities (202).

Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC)

Thousands of underprivileged cancer patients received free comprehensive care at the clinic in 1989 (203). It is a humanitarian institute in Punjab formed by former cricketer and Prime Minister of Pakistan Imran Khan and is primarily sponsored by donations from friends and well-wishers (203). It aims to serve as a model organization for improving the welfare of cancer patients *via* the use of current preventive and palliative therapy techniques for all cancer patients, regardless of their ability to pay, health care professionals and public education, and cancer research (203).

Surgical Oncology Society Pakistan (SOS-PK)

SOS-PK is a professional society promoting cancer patient treatment in Pakistan by enabling interaction and lifelong learning. Pakistan is subjected to a substantial illness load (204). The SOS-PK is Pakistan's leading national cancer surgeons' representative organization. It is a member of the ESSO (European Society of Surgical Oncology) and the Society of Surgical Oncology and USA's Global Cancer Surgery Leadership Forum Initiative (204).

The goals of SOS-PK (founded in Lahore in 2008) are to promote the advancement of cancer surgery education and training by improving communications among surgeons with a primary interest in the subject (204). SOS-PK accomplishes this by hosting conferences and symposia across the country to advocate for the highest possible quality of cancer care for patients, highlighting the necessity of a multi-sectoral approach and campaigning for the establishment of comprehensive cancer hospitals that provide all treatment under one roof and by raising public awareness, it is expected to encourage cancer prevention and early detection among the general population, health administrators, and healthcare providers (204).

Food and Nutrition

One of the most critical determinants of human resource quality is nutritional adequacy. Cancer remains a serious public health problem despite significant advancements in technology in the health and other medical industries (205). The National Action Plan for Non-Communicable Disease Prevention, Control, and Health Promotion in Pakistan (NAP-NCD) combines cancer prevention and control with a comprehensive Non-communicable Diseases (NCD) prevention framework that includes cross-cutting risks such as tobacco and food and physical activity (206). By marking cancer days and world cancer day, the PHRC hopes to improve public awareness of six common diseases of body parts (oral, lung, and liver, breast, cervical, and blood/bone marrow; schedule attached). An education pamphlet on the risk factors and prevention of common cancers has been developed in English and Urdu to easily comprehend common cancers in Pakistan. A deficiency of macronutrients and micronutrients, such as iron (207), vitamin A (208), vitamin C (209), and vitamin D (210), causes cancer (207). The major causes of such deficiencies are insufficient bioavailability and insufficient food intake. A balanced diet ensures sufficient nutrients for a healthy life (211). Malnutrition affects millions worldwide due to inadequate food intake and disease (212). Pakistan is attempting to address health-related concerns by implementing various food and nutrition initiatives.

Food and Nutrition Society Gilgit Baltistan (FNSGB)

FNSGB is a platform for giving people awareness about the basic needs of nutrition and healthcare. Public health issues related to food, nutrition, and health-related disorders are the major handling areas of this society (59). It gives policy and future planning for food and nutrition programs and dietary guidelines for the population on the local level (98). Delivering different campaigns having information about the cancer risk factor and their relation to the dietary patterns and food is the primary core field of this society (6, 62, 98).

Strategies to Avoid Cancer

Anti-smoking regulations in public places have only recently gained traction; even then, they are not being adequately enforced

(213). To manage this problem, adequate measures such as anti-smoking education must be used. Advertisements have been discovered to play a substantial role in promoting smoking (214, 215). In Pakistan, regulations prohibiting such advertising techniques were enacted. In Pakistan, two principal regulations oversee tobacco control (216). Using the powers conferred by the two pieces of law. The first necessary regulation is the cigarettes (printing of warning) ordinance of 1979 (Ordinance No. LXXIII of 1979) (217). This essentially requires health warnings to be included on cigarette product packaging. Statutory regulatory orders established the regulations for publishing warnings (SROs) 86(KE)/2009 (218). The initial warning wording and graphics are established by SRO 87(E)/2009 (219). The second necessary regulation is the Ban of Smoking in Enclosed Places and Protection of Non-smokers Health Law, approved in 2002 (Ordinance No. LXXIV of 2002) (220). It controls a variety of facets of tobacco control, including public smoking prohibitions, sales to minors, cigarette advertisements, marketing, and finance. The Committee on Tobacco Advertisement Guidelines was formed by SRO 655(I)/2003 in tobacco advertising, marketing, and finance (221). Despite predictions that increasing the price of cigarettes by 10% will result in a 4.8% immediate drop and a long-term reduction of 11.7% in cigarette smoking, business economics are proving to be a pivotal hurdle to tobacco control (97). SRO 2019 will display a 60% pictorial warning label on cigarette boxes.

CONCLUSION

This review focuses on cancer and its prevalence across the globe, as well as in Pakistan. Cancer is one of the leading causes of mortality around the globe. In 2025, an estimated 19.3 million more cancer cases [18.1 million excluding non-melanoma skin cancer (NMSC) and basal carcinoma] and 10 million additional cancer deaths (9.9 million excluding non-melanoma skin cancers and squamous cell carcinoma) will have been reported globally. Several cancers are also becoming more common in Pakistan. Pakistan's population is 220.9 million. According to GLOBACAN 2020, In Pakistan, there have been 178,388 new cancer cases, 117,149 cancer deaths, and 329,547 overall cancers cases found in five years. Cancer may be caused by poor nutrition, and harmful behaviors such as eating more junk food, adulterating infectious substances, gutkha, and paan. Pakistan is nutritionally deficient in vitamin D, vitamin A, zinc, and iron, according to the national nutrition survey report of 2018 (175). These nutritional deficiencies are the contributing causes of cancer. The use of gutkha, paan, fast foods and adulteration of foods are common culprits for cancer. When it comes to cancer therapy, Pakistan lacks proper policies and strategies. Different governmental and non-governmental organizations are working which are nonetheless making a substantial contribution to the health sector by applying nuclear and other cutting edge technology for cancer diagnosis and treatment. The national action plan also works on cancer prevention measures for NAP-NCD and other groups.

This is a difficult aim for Pakistan to achieve, and it will need dedication from all levels of society. Many characteristics connected to cancer burden discussed here assist to highlight features of cancer epidemiology that may be used to drive intervention programs and promote cancer determinants and outcomes research. Current data on cancer burden will be required for the establishment of national NCD action and cancer control plans, and cancer control measures must be prioritized based on local requirements. Annual updates on the cancer burden will be published in response to this demand.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Godet I, Gilkes DM. BRCA1 and BRCA2 mutations and treatment strategies for breast cancer. *Integr Cancer Sci Ther*. (2017) 4:1–7. doi: 10.15761/ICST.1000228
2. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. *Best Pract Res Clin Gastroenterol.* (2006) 20:197–209. doi: 10.1016/j.bpg.2005.10.001
3. Kampa M, Castanas E. Human health effects of air pollution. *Environ. Pollut.* (2008) 151:362–7. doi: 10.1016/j.envpol.2007.06.012
4. Duell EJ. Epidemiology and potential mechanisms of tobacco smoking and heavy alcohol consumption in pancreatic cancer. *Mol Carcinog.* (2012) 51:40–52. doi: 10.1002/mc.20786
5. Williams PD, Piamjariyakul U, Ducey K, Badura J, Boltz KD, Olberding K, et al. Cancer treatment, symptom monitoring, and self-care in adults: pilot study. *Cancer Nurs.* (2006) 29:347–55. doi: 10.1097/00002820-200609000-00001
6. Ali A, Mughal H, Ahmad N, Babar Q, Saeed A, Khalid W, et al. Novel therapeutic drug strategies to tackle immune-oncological challenges faced by cancer patients during COVID-19. *Expert Rev Anticancer Ther.* (2021) 21:1371–83. doi: 10.1080/14737140.2021.1991317
7. WebMD Cancer Center. *Understanding Cancer – the Basics.* (2020). Available online at: https://www.webmd.com/cancer/guide/understanding-cancer-basics (accessed March 12, 2022).
8. Elliott P, Shaddick G, Douglass M, de Hoogh K, Briggs DJ, Toledano MB. Adult cancers near high-voltage overhead power

lines. *Epidemiology*. (2013) 24:184–90. doi: 10.1097/EDE.0b013e31827e95b9

9. Center SKMCHaR. *Cancer Statistics*. (2020). Available online at: https://shaukatkhanum.org.pk/health-care-professionals-researchers/cancer-statistics/ (accessed March 16, 2022).
10. Turcotte LM, Neglia JP, Reulen RC, Ronckers CM, Van Leeuwen FE, Morton LM, et al. Risk, risk factors, and surveillance of subsequent malignant neoplasms in survivors of childhood cancer: a review. *J Clin Oncol*. (2018) 36:2145. doi: 10.1200/JCO.2017.76.7764
11. Anis R, Gaballah K. Oral cancer in the UAE: a multicenter, retrospective study. *Libyan J Med*. (2013) 8:21782. doi: 10.3402/ljm.v8i0.21782
12. De Angelis R, Francisci S, Baili P, Marchesi F, Roazzi P, Belot A, et al. The EUROCARE-4 database on cancer survival in Europe: data standardisation, quality control and methods of statistical analysis. *Eur J Cancer*. (2009) 45:909–30. doi: 10.1016/j.ejca.2008.11.003
13. Hochberg J, Waxman IM, Kelly KM, Morris E, Cairo MS. Adolescent non-Hodgkin lymphoma and Hodgkin lymphoma: state of the science. *Br J Haematol*. (2009) 144:24–40. doi: 10.1111/j.1365-2141.2008.07393.x
14. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends-an update. *Cancer Epidemiol Prevent Biomark*. (2016) 25:16–27. doi: 10.1158/1055-9965.EPI-15-0578
15. Barbeiro S, Atalaia-Martins C, Marcos P, Gonçalves C, Cotrim I, Vasconcelos H, et al. case series of anal carcinoma misdiagnosed as idiopathic chronic anal fissure. *GE-Portug J Gastroenterol*. (2017) 24:227–31. doi: 10.1159/000452869
16. American Cancer Society. *Cancer Staging*. (2021). Available online at: https://www.cancer.org/treatment/understanding-your-diagnosis/staging.html (accessed March 17, 2022).
17. Feitelson MA, Arzumanyan A, Kulathinal RJ, Blain SW, Holcombe RF, Mahajna J, et al. Sustained proliferation in cancer: mechanisms and novel therapeutic targets. *Semin Cancer Biol*. (2015) 35(Suppl.):S25–54. doi: 10.1016/j.semcancer.2015.02.006
18. Labi V, Erlacher M. How cell death shapes cancer. *Cell Death Dis*. (2015) 6:e1675.e. doi: 10.1038/cddis.2015.20
19. Egelund R, Petersen H. The plasminogen activation system in tumor growth, invasion, and metastasis. *Cell Mol Life Sci*. (2000) 57:25–40. doi: 10.1007/s000180050497
20. Marx RE, Ehler WJ, Tayapongsak P, Pierce LW. Relationship of oxygen dose to angiogenesis induction in irradiated tissue. *Am J Surg*. (1990) 160:519–24. doi: 10.1016/S0002-9610(05)81019-0
21. Gentry M. World Cancer Research Fund International (WCRF). *Impact*. (2017) 2017:32–3. doi: 10.21820/23987073.2017.4.32
22. World Cancer Research Fund International. *Research Findings*. (2021). Available online at: https://www.wcrf.org/research-we-fund/research-findings/ (accessed March 22, 2022).
23. Hartwell LH, Unger MW. Unequal division in Saccharomyces cerevisiae and its implications for the control of cell division. *J Cell Biol*. (1977) 75:422–35. doi: 10.1083/jcb.75.2.422
24. Sumner AT. *Chromosomes*: *Organization and Function*. Malden, MA: John Wiley & Sons (2008).
25. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature*. (2004) 432:316–23. doi: 10.1038/nature03097
26. Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*. (2006) 160:1–40. doi: 10.1016/j.cbi.2005.12.009
27. Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. *Cancer Res*. (2008) 68:6863–72. doi: 10.1158/0008-5472.CAN-08-2852
28. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660
29. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: an overview. *Int J Cancer*. (2021) 149:778–89. doi: 10.1002/ijc.33588
30. Lei S, Zheng R, Zhang S, Wang S, Chen R, Sun K, et al. Global patterns of breast cancer incidence and mortality: a population-based cancer registry data analysis from 2000 to 2020. *Cancer Commun*. (2021) 41:1183–94. doi: 10.1002/cac2.12207
31. Hussain I, Majeed A, Rasool MF, Hussain M, Imran I, Ullah M, et al. Knowledge, attitude, preventive practices and perceived barriers to screening about colorectal cancer among university students of newly merged district, Kpk, Pakistan-A cross-sectional study. *J Oncol Pharm Pract*. (2021) 27:359–67. doi: 10.1177/1078155220922598
32. Rashid A, Aqeel M, Malik B, Salim S. The prevalence of psychiatric disorders in breast cancer patients; a cross-sectional study of breast cancer patients experience in Pakistan. *Nature*. (2021) 1:1–7. doi: 10.47391/NNJP.01
33. Sohail S, Alam SN. Breast cancer in Pakistan-awareness and early detection (2007). Available online at: https://ecommons.aku.edu/cgi/viewcontent.cgi?article=1449&context=pakistan_fhs_mc_radiol
34. Sarwar MR, Saqib A. Cancer prevalence, incidence and mortality rates in Pakistan in 2012. *Cogent Med*. (2017) 4:1288773. doi: 10.1080/2331205X.2017.1288773
35. Anwar N, Pervez S, Chundriger Q, Awan S, Moatter T, Ali TS. Oral cancer: Clinicopathological features and associated risk factors in a high risk population presenting to a major tertiary care center in Pakistan. *PLoS ONE*. (2020) 15:e0236359. doi: 10.1371/journal.pone.0236359
36. Niaz K, Maqbool F, Khan F, Bahadar H, Hassan FI, Abdollahi M. Smokeless tobacco (paan and gutkha) consumption, prevalence, and contribution to oral cancer. *Epidemiol Health*. (2017) 39:e2017009. doi: 10.4178/epih.e2017009
37. Siddiqi K, Shah S, Abbas SM, Vidyasagaran A, Jawad M, Dogar O, et al. Global burden of disease due to smokeless tobacco consumption in adults: analysis of data from 113 countries. *BMC Med*. (2015) 13:194. doi: 10.1186/s12916-015-0424-2
38. Aktas A, Walsh D, Galang M, O'Donoghue N, Rybicki L, Hullihen B, et al. Underrecognition of malnutrition in advanced cancer: the role of the dietitian and clinical practice variations. *Am J Hosp Palliat Med*. (2017) 34:547–55. doi: 10.1177/1049909116639969
39. Bossi P, Delrio P, Mascheroni A, Zanetti M. The spectrum of malnutrition/cachexia/sarcopenia in oncology according to different cancer types and settings: a narrative review. *Nutrients*. (2021) 13:1980. doi: 10.3390/nu13061980
40. Ravasco P. Nutrition in cancer patients. *J Clin Med*. (2019) 8:1211. doi: 10.3390/jcm8081211
41. Planas M, Álvarez-Hernández J, León-Sanz M, Celaya-Pérez S, Araujo K, García de. Lorenzo A. Prevalence of hospital malnutrition in cancer patients: a sub-analysis of the PREDyCES® study. *Support Care Cancer*. (2016) 24:429–35. doi: 10.1007/s00520-015-2813-7
42. Capra S, Ferguson M, Ried K. Cancer: impact of nutrition intervention outcome-nutrition issues for patients. *Nutrition*. (2001) 17:769–72. doi: 10.1016/S0899-9007(01)00632-3
43. Bourke CD, Berkley JA, Prendergast AJ. Immune dysfunction as a cause and consequence of malnutrition. *Trends Immunol*. (2016) 37:386–98. doi: 10.1016/j.it.2016.04.003
44. Barker LA, Gout BS, Crowe TC. Hospital malnutrition: prevalence, identification and impact on patients and the healthcare system. *Int J Environ Res Public Health*. (2011) 8:514–27. doi: 10.3390/ijerph8020514
45. Dasarathy S, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. *J Hepatol*. (2016) 65:1232–44. doi: 10.1016/j.jhep.2016.07.040
46. Freijer K, Tan SS, Koopmanschap MA, Meijers JM, Halfens RJ, Nuijten MJ. The economic costs of disease related malnutrition. *Clin Nutr*. (2013) 32:136–41. doi: 10.1016/j.clnu.2012.06.009
47. Rasheed S, Woods RT. Malnutrition and quality of life in older people: a systematic review and meta-analysis. *Ageing Res Rev*. (2013) 12:561–6. doi: 10.1016/j.arr.2012.11.003
48. Lummaa V, Clutton-Brock T. Early development, survival and reproduction in humans. *Trends Ecol Evol*. (2002) 17:141–7. doi: 10.1016/S0169-5347(01)02414-4
49. Shiferaw B, Smale M, Braun H-J, Duveiller E, Reynolds M, Muricho G. Crops that feed the world 10. Past successes and future challenges to the

role played by wheat in global food security. *Food Secur.* (2013) 5:291–317. doi: 10.1007/s12571-013-0263-y

50. Holmboe-Ottesen G, Wandel M. Changes in dietary habits after migration and consequences for health: a focus on South Asians in Europe. *Food Nutr Res.* (2012) 56:18891. doi: 10.3402/fnr.v56i0.18891
51. Ali A, Shaukat H, Ahmed M, Bostani A, Hussain S. Relation of electrical stimulation to meat standard. *Vet Sci.* (2021) 7:42–51. doi: 10.17582/journal.vsrr/2021.7.1.42.51
52. Mellin-Olsen T, Wandel M. Changes in food habits among Pakistani immigrant women in Oslo, Norway. *Ethn Health.* (2005) 10:311–39. doi: 10.1080/13557850500145238
53. World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR). *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective.* Washington, DC: WCRF/AICR (2007).
54. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry.* (2001) 56:5–51. doi: 10.1016/S0031-9422(00)00316-2
55. Clements Jr RS, Darnell B. Myo-inositol content of common foods: development of a high-myo-inositol diet. *Am J Clin Nutr.* (1980) 33:1954–67. doi: 10.1093/ajcn/33.9.1954
56. Hecht SS. Inhibition of carcinogenesis by isothiocyanates. *Drug Metab Rev.* (2000) 32:395–411. doi: 10.1081/DMR-100102342
57. Hecht SS, Kenney PM, Wang M, Upadhyaya P. Dose-response study of myo-inositol as an inhibitor of lung tumorigenesis induced in A/J mice by benzo [a] pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Lett.* (2001) 167:1–6. doi: 10.1016/S0304-3835(01)00454-2
58. Maruthanila V, Poornima J, Mirunalini S. Attenuation of carcinogenesis and the mechanism underlying by the influence of indole-3-carbinol and its metabolite 3, 3′-diindolylmethane: a therapeutic marvel. *Adv Pharmacol Sci.* (2014) 2014:832161. doi: 10.1155/2014/832161
59. Khalid W, Ali A, Arshad MS, Afzal F, Akram R, Siddeeg A, et al. Nutrients and bioactive compounds of *Sorghum bicolor* L. used to prepare functional foods: a review on the efficacy against different chronic disorders. *Int J Food Proper.* (2022) 25:1045–62. doi: 10.1080/10942912.2022.2071293
60. Rokayya S, Li C-J, Zhao Y, Li Y, Sun C-H. Cabbage (*Brassica oleracea* L. var capitata) phytochemicals with antioxidant and anti-inflammatory potential Asian Pacific. *J Cancer Prevent.* (2013) 14:6657–62. doi: 10.7314/APJCP.2013.14.11.6657
61. Craig WJ. Nutrition concerns and health effects of vegetarian diets. *Nutr Clin Pract.* (2010) 25:613–20. doi: 10.1177/0884533610385707
62. Babar Q, Ali A, Saeed A, Tahir MF. Novel treatment strategy against COVID-19 through anti-inflammatory, antioxidant and immunostimulatory properties of the b vitamin complex. In: *B-Complex Vitamins: Sources, Intakes and Novel Applications*. Intechopen (2021). doi: 10.5772/intechopen.100251
63. Farag MA, Motaal AAA. Sulforaphane composition, cytotoxic and antioxidant activity of crucifer vegetables. *J Adv Res.* (2010) 1:65–70. doi: 10.1016/j.jare.2010.02.005
64. Abbaoui B, Lucas CR, Riedl KM, Clinton SK, Mortazavi A. Cruciferous vegetables, isothiocyanates, and bladder cancer prevention. *Mol Nutr Food Res.* (2018) 62:1800079. doi: 10.1002/mnfr.201800079
65. Liang H, Wei Y, Li R, Cheng L, Yuan Q, Zheng F. Intensifying sulforaphane formation in broccoli sprouts by using other cruciferous sprouts additions. *Food Sci Biotechnol.* (2018) 27:957–62. doi: 10.1007/s10068-018-0347-8
66. Gautam S, Saxena S, Kumar S. Fruits and vegetables as dietary sources of antimutagens. *J Food Chem Nanotechnol.* (2016) 2:97–114. doi: 10.17756/jfcn.2016-018
67. Banerjee S, Kong D, Wang Z, Bao B, Hillman GG, Sarkar FH. Attenuation of multi-targeted proliferation-linked signaling by 3, 3′-diindolylmethane (DIM): from bench to clinic. *Mutat Res Rev.* (2011) 728:47–66. doi: 10.1016/j.mrrev.2011.06.001
68. Arora S, Vig AP. Inhibition of DNA oxidative damage and antimutagenic activity by dichloromethane extract of *Brassica rapa* var. rapa L seeds. *Indus Crops Prod.* (2015) 74:585–91. doi: 10.1016/j.indcrop.2015.05.038
69. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: promising anticancer agents. *Med Res Rev.* (2003) 23:519–34. doi: 10.1002/med.10033
70. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables-the millennium's health. *Int J Food Sci Technol.* (2001) 36:703–25. doi: 10.1046/j.1365-2621.2001.00513.x
71. Chaudhary P, Sharma A, Singh B, Nagpal AK. Bioactivities of phytochemicals present in tomato. *J Food Sci Technol.* (2018) 55:2833–49. doi: 10.1007/s13197-018-3221-z
72. D'Archivio M, Santangelo C, Scazzocchio B, Varì R, Filesi C, Masella R, et al. Modulatory effects of polyphenols on apoptosis induction: relevance for cancer prevention. *Int J Mol Sci.* (2008) 9:213–28. doi: 10.3390/ijms9030213
73. Das Gupta S, Suh N. Tocopherols in cancer: an update. *Mol Nutr Food Res.* (2016) 60:1354–63. doi: 10.1002/mnfr.201500847
74. Eggersdorfer M, Wyss A. Carotenoids in human nutrition and health. *Arch Biochem Biophys.* (2018) 652:18–26. doi: 10.1016/j.abb.2018.06.001
75. Schmidt KM, Haddad EN, Sugino KY, Vevang KR, Peterson LA, Koratkar R, et al. Dietary and plasma carotenoids are positively associated with alpha diversity in the fecal microbiota of pregnant women. *J Food Sci.* (2021) 86:602–13. doi: 10.1111/1750-3841.15586
76. Brewczyński A, Jabłońska B, Kentnowski M, Mrowiec S, Składowski K, Rutkowski T. The association between carotenoids and head and neck cancer risk. *Nutrients.* (2022) 14:88. doi: 10.3390/nu14010088
77. van den Brandt PA. Red meat, processed meat, and other dietary protein sources and risk of overall and cause-specific mortality in The Netherlands Cohort Study. *Eur J Epidemiol.* (2019) 34:351–69. doi: 10.1007/s10654-019-00483-9
78. Saqib SE, Kuwornu JK, Panezia S, Ali U. Factors determining subsistence farmers' access to agricultural credit in flood-prone areas of Pakistan. *Kasetsart J Soc Sci.* (2018) 39:262–8. doi: 10.1016/j.kjss.2017.06.001
79. Staal SJ, Nin Pratt A, Jabbar M. Dairy development for the resource poor. In: *Part 3: Pakistan and India Dairy Development Case Studies FAO/PPLPI Working Paper*. Rome: Food and Agriculture Organization (2008).
80. Vorley B, Lundy M, MacGregor J. *Business Models That Are Inclusive of Small Farmers*. Agro-industries for Development. Wallingford: CABI for FAO and UNIDO (2009). p. 186–222. doi: 10.1079/9781845935764.0186
81. Tahir MN, Riaz R, Bilal M, Nouman HM. Current standing and future challenges of dairying in Pakistan: a status update. In: Javed K, editor. *Milk Production, Processing and Marketing*. London (2019). p. 1–117.
82. Awan A, Naseer M, Iqbal A, Ali M, Iqbal R, Iqbal F. A study on chemical composition and detection of chemical adulteration in tetra pack milk samples commercially available in Multan. *Pakistan J Pharm Sci.* (2014) 27:183–6. Available online at: http://www.pjps.pk/wp-content/uploads/pdfs/27/1/Paper-27.pdf
83. Jalil H, Rehman HU, Sial MH, Hussain SS. Analysis of milk production system in Peri-urban areas of Lahore (Pakistan): a case study. *Pakistan Econ Soc Rev.* (2009) 47:229–42. Available online at: http://www.jstor.org/stable/25825354
84. Motta TC, Hoff R, Barreto F, Andrade R, Lorenzini D, Meneghini L, et al. Detection and confirmation of milk adulteration with cheese whey using proteomic-like sample preparation and liquid chromatography-electrospray-tandem mass spectrometry analysis. *Talanta.* (2014) 120:498–505. doi: 10.1016/j.talanta.2013.11.093
85. Javed K, Afzal M, Sattar A, Mirza R. Environmental factors affecting milk yield in Friesian cows in Punjab, Pakistan. *Pak Vet J.* (2004) 24:58–61. Available online at: http://www.pvj.com.pk/pdf-files/24_2/58-61.pdf
86. Handford CE, Campbell K, Elliott CT. Impacts of milk fraud on food safety and nutrition with special emphasis on developing countries. *Comprehens Rev Food Sci Food Saf.* (2016) 15:130–42. doi: 10.1111/1541-4337.12181
87. KC B, Schultz B, McIndoe I, Rutter H, Dark A, Prasad K, et al. Impacts of dairy farming systems on water quantity and quality in Brazil, Ethiopia, Nepal, New Zealand and the USA. *Irrigat Drain.* (2020) 69:944–55. doi: 10.1002/ird.2486
88. Singh P, Gandhi N. Milk preservatives and adulterants: processing, regulatory and safety issues. *Food Rev Int.* (2015) 31:236–61. doi: 10.1080/87559129.2014.994818
89. Boada LD, Henríquez-Hernández LA, Luzardo O. The impact of red and processed meat consumption on cancer and other health outcomes: Epidemiological evidences. *Food Chem Toxicol.* (2016) 92:236–44. doi: 10.1016/j.fct.2016.04.008

90. Peleteiro B, Lopes C, Figueiredo C, Lunet N. Salt intake and gastric cancer risk according to *Helicobacter pylori* infection, smoking, tumour site and histological type. *Br J Cancer.* (2011) 104:198–207. doi: 10.1038/sj.bjc.6605993
91. Solans M, Castelló A, Benavente Y, Marcos-Gragera R, Amiano P, Gracia-Lavedan E, et al. Adherence to the Western, Prudent, and Mediterranean dietary patterns and chronic lymphocytic leukemia in the MCC-Spain study. *Haematologica.* (2018) 103:1881. doi: 10.3324/haematol.2018.192526
92. Wu AH, Pike MC, Stram DO. Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. *J Natl Cancer Inst.* (1999) 91:529–34. doi: 10.1093/jnci/91.6.529
93. Black HS, Chan JT. Suppression of ultraviolet light-induced tumor formation by dietary antioxidants. *J Invest Dermatol.* (1975) 65:412–4. doi: 10.1111/1523-1747.ep12607661
94. Carbamate E. *Volume 96: Alcohol Consumption and Ethyl Carbamate*. World Health Organization International Agency for Research on Cancer (2010). p. 632–714. Available online at: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Alcohol-Consumption-And-Ethyl-Carbamate-2010
95. Mukherjee P, Seyfried T. Metabolic targeting of brain cancer. *Nutr Metab (Lond).* (2005) 2:30. doi: 10.1186/1743-7075-2-30
96. Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. *Gastroenterology.* (2004) 127:S97–103. doi: 10.1053/j.gastro.2004.09.021
97. Ahmad SA, Ahmed M, Qadir MA, Shafiq MI, Batool N, Nosheen N, et al. Quantitation and risk assessment of chemical adulterants in milk using UHPLC coupled to photodiode array and differential refractive index detectors. *Food Anal Methods.* (2016) 9:3367–76. doi: 10.1007/s12161-016-0534-2
98. Ali A, Ain Q, Saeed A, Khalid W, Ahmed M, Bostani A. Bio-molecular characteristics of whey proteins with relation to inflammation. In: *New Advances in the Dairy Industry*. Intechopen (2021). doi: 10.5772/intechopen.99220
99. Patel PK, Patel SK, Dixit S, Rathore R. Gastritis and peptic ulcer diseases in dogs: A review. *Int J Curr Microbiol App Sci.* (2018) 7:2475–501. doi: 10.20546/ijcmas.2018.703.288
100. Xiao A, Yang S, Iqbal Q. Factors affecting purchase intentions in generation Y: an empirical evidence from fast food industry in Malaysia. *Administr Sci.* (2018) 9:4. doi: 10.3390/admsci9010004
101. Musaiger AO. Consumption, health attitudes and perception toward fast food among Arab consumers in Kuwait: gender differences. *Glob J Health Sci.* (2014) 6:136. doi: 10.5539/gjhs.v6n6p136
102. Tannock S. *Youth at Work: The Unionized Fast-Food and Grocery Workplace*. Philadelphia, PA: Temple University Press (2001).
103. Harris JL, Schwartz MB, Brownell KD. *Evaluating Fast Food Nutrition and Marketing to Youth.* New Haven, CT: Yale Rudd Center for Food Policy & Obesity (2010).
104. Baig AK, Saeed M. Review of trends in fast food consumption. *Eur J Econ Finan Administr Sci.* (2012) 48:77–85. Available online at: https://www.researchgate.net/profile/Munazza-Saeed-3/publication/266627067_Review_of_Trends_in_Fast_Food_Consumption/links/543652160cf2dc341db2fa61/Review-of-Trends-in-Fast-Food-Consumption.pdf
105. Blisard WN, Jolliffe D. *Let's Eat Out: Americans Weigh Taste, Convenience and Nutrition*. US Department of Agriculture, Economic Research Service (2006).
106. Cohen DA, Babey SH. Contextual influences on eating behaviours: heuristic processing and dietary choices. *Obes Rev.* (2012) 13:766–79. doi: 10.1111/j.1467-789X.2012.01001.x
107. Mead E, Gittelsohn J, Kratzmann M, Roache C, Sharma S. Impact of the changing food environment on dietary practices of an Inuit population in Arctic Canada. *J Hum Nutr Dietet.* (2010) 23:18–26. doi: 10.1111/j.1365-277X.2010.01102.x
108. James D. Factors influencing food choices, dietary intake, and nutrition-related attitudes among African Americans: application of a culturally sensitive model. *Ethn Health.* (2004) 9:349–67. doi: 10.1080/1355785042000285375
109. Harrington RJ, Ottenbacher MC, Kendall K. Fine-dining restaurant selection: Direct and moderating effects of customer attributes. *J Foodservice Bus Res.* (2011) 14:272–89. doi: 10.1080/15378020.2011.594388
110. Anand R. A. study of determinants impacting consumers food choice with reference to the fast food consumption in India. *Soc Bus Rev.* (2011) 6:176–187. doi: 10.1108/17465681111143993
111. Viner RM, Ozer EM, Denny S, Marmot M, Resnick M, Fatusi A, et al. Adolescence and the social determinants of health. *Lancet.* (2012) 379:1641–52. doi: 10.1016/S0140-6736(12)60149-4
112. Fuhrman J. The hidden dangers of fast and processed food. *Am J Lifestyle Med.* (2018) 12:375–81. doi: 10.1177/1559827618766483
113. Yahya F, Zafar R, Shafiq S. Trend of fast food consumption and its effect on Pakistani society. *Food Sci Qual Manage.* (2013) 11:1–7. Available online at: https://core.ac.uk/download/pdf/234683616.pdf
114. Yan Y. *Of Hamburger and Social Space: Consuming.* Beijing: McDonald's (2000).
115. Boutelle KN, Fulkerson JA, Neumark-Sztainer D, Story M, French SA. Fast food for family meals: relationships with parent and adolescent food intake, home food availability and weight status. *Public Health Nutr.* (2007) 10:16–23. doi: 10.1017/S136898000721794X
116. Abraham S, Martinez M, Salas G, Smith J. College student's perception of risk factors related to fast food consumption and their eating habits. *J Nutr Hum Health.* (2018) 2:18–21. doi: 10.35841/nutrition-human-health.2.1.18-21
117. Meng H, Hu W, Chen Z, Shen Y. Fruit and vegetable intake and prostate cancer risk: a meta-analysis. *Asia-Pac J Clin Oncol.* (2014) 10:133–40. doi: 10.1111/ajco.12067
118. Castelló A, Boldo E, Amiano P, Castaño-Vinyals G, Aragonés N, Gómez-Acebo I, et al. Mediterranean dietary pattern is associated with low risk of aggressive prostate cancer: MCC-Spain Study. *J Urol.* (2018) 199:430–7. doi: 10.1016/j.juro.2017.08.087
119. Dieli-Conwright CM, Lee K, Kiwata JL. Reducing the risk of breast cancer recurrence: an evaluation of the effects and mechanisms of diet and exercise. *Curr Breast Cancer Rep.* (2016) 8:139–50. doi: 10.1007/s12609-016-0218-3
120. Xu X, Li J, Wang X, Wang S, Meng S, Zhu Y, et al. Tomato consumption and prostate cancer risk: a systematic review and meta-analysis. *Sci Rep.* (2016) 6:1–8. doi: 10.1038/srep37091
121. Iqbal R, Khan AH. Possible causes of vitamin D deficiency (VDD) in Pakistani population residing in Pakistan. *J Pak Med Assoc.* (2010) 60:1–2. Available online at: https://www.jpma.org.pk/article-details/1888
122. Khan H, Ansari M, Waheed U, Farooq N. Prevalence of vitamin D deficiency in general population of Islamabad, Pakistan. *Ann Pak Inst Med Sci.* (2013) 9:45–7. Available online at: https://www.researchgate.net/publication/269692575_Prevalence_of_Vitamin_D_Deficiency_in_general_population_of_Islamabad_Pakistan
123. Chlebowski RT, Johnson KC, Kooperberg C, Pettinger M, Wactawski-Wende J, Rohan T, et al. Calcium plus vitamin D supplementation and the risk of breast cancer. *J Natl Cancer Instit.* (2008) 100:1581–91. doi: 10.1093/jnci/djn360
124. Tworoger SS, Lee I-M, Buring JE, Rosner B, Hollis BW, Hankinson SE. Plasma 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D and risk of incident ovarian cancer. *Cancer Epidemiol Prevent Biomark.* (2007) 16:783–8. doi: 10.1158/1055-9965.EPI-06-0981
125. Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, et al. Plasma 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Prevent Biomark.* (2005) 14:1991–7. doi: 10.1158/1055-9965.EPI-04-0722
126. Gupta PC, Murti P, Bhonsle R. Epidemiology of cancer by tobacco products and the significance of TSNA. *Crit Rev Toxicol.* (2017) 26:183-98. doi: 10.3109/10408449609017930
127. Whitehead TP, Havel C, Metayer C, Benowitz NL, Jacob P III. Tobacco alkaloids and tobacco-specific nitrosamines in dust from homes of smokeless tobacco users, active smokers, and nontobacco users. *Chem Res Toxicol.* (2015) 28:1007–14. doi: 10.1021/acs.chemrestox.5b00040
128. Banerjee SC, Ostroff JS, Bari S, D'Agostino TA, Khera M, Acharya S, et al. Gutka and Tambaku paan use among South Asian immigrants:

a focus group study. *J Immigr Minor Health.* (2014) 16:531–9. doi: 10.1007/s10903-013-9826-4
129. Shah G, Chaturvedi P, Vaishampayan S. Arecanut as an emerging etiology of oral cancers in India. *Indian J Med Paediatr Oncol.* (2012) 33:71. doi: 10.4103/0971-5851.99726
130. Pillai R, Balaram P, Reddiar KS. Pathogenesis of oral submucous fibrosis. Relationship to risk factors associated with oral cancer. *Cancer.* (1992) 69:2011–20. doi: 10.1002/1097-0142(19920415)69:8<2011::AID-CNCR2820690802>3.0.CO;2-B
131. Thomas G, Hashibe M, Jacob BJ, Ramadas K, Mathew B, Sankaranarayanan R, et al. Risk factors for multiple oral premalignant lesions. *Int J Cancer.* (2003) 107:285–91. doi: 10.1002/ijc.11383
132. Zhao L, Mbulo L, Twentyman E, Palipudi K, King BA. Disparities in smokeless tobacco use in Bangladesh, India, and Pakistan: findings from the global adult tobacco survey, 2014-2017. *PLoS ONE.* (2021) 16:e0250144. doi: 10.1371/journal.pone.0250144
133. Petti S. Lifestyle risk factors for oral cancer. *Oral Oncol.* (2009) 45:340–50. doi: 10.1016/j.oraloncology.2008.05.018
134. Rao SVK, Mejia G, Roberts-Thomson K, Logan R. Epidemiology of oral cancer in Asia in the past decade-an update (2000-2012). *Asian Pac J Cancer Prevent.* (2013) 14:5567–77. doi: 10.7314/APJCP.2013.14.10.5567
135. García-Martín JM, Varela-Centelles P, González M, Seoane-Romero JM, Seoane J, García-Pola MJ. Epidemiology of oral cancer. *Oral Cancer Detect.* (2019) 81–93. doi: 10.1007/978-3-319-61255-3_3
136. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer.* (2010) 127:2893–917. doi: 10.1002/ijc.25516
137. Qidwai W, Saleheen D, Saleem S, Andrades M, Azam I. Are our people health conscious? Results of a patients survey in Karachi, Pakistan. *J Ayub Med Coll.* (2003) 15:10.
138. Mahmood Z. Smoking and chewing habits of people of Karachi—1981. *J Pak Med Assoc.* (1982) 32:34–7.
139. Khawaja M, Mazahir S, Majeed A, Malik F, Merchant K, Maqsood M, et al. Knowledge, attitude and practices of a Karachi slum population regarding the role of products of betel, areca and smokeless tobacco in the etiology of head & neck cancers. *J Pak Med Assoc.* (2005) 55:S41.
140. Shah S, Merchant A, Luby S, Chotani R. Addicted schoolchildren: prevalence and characteristics of areca nut chewers among primary school children in Karachi, Pakistan. *J Paediatr Child Health.* (2002) 38:507–10. doi: 10.1046/j.1440-1754.2002.00040.x
141. Mazahir S, Malik R, Maqsood M, Merchant KA, Malik F, Majeed A, et al. Socio-demographic correlates of betel, areca and smokeless tobacco use as a high risk behavior for head and neck cancers in a squatter settlement of Karachi, Pakistan. *Subst Abuse Treat Prev Policy.* (2006) 1:1–6. doi: 10.1186/1747-597X-1-10
142. Javed F, Altamash M, Klinge B, Engström P-E. Periodontal conditions and oral symptoms in gutka-chewers with and without type 2 diabetes. *Acta Odontol Scand.* (2008) 66:268–73. doi: 10.1080/00016350802286725
143. Javed F, Chotai M, Mehmood A, Almas K. Oral mucosal disorders associated with habitual gutka usage: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol.* (2010) 109:857–64. doi: 10.1016/j.tripleo.2009.12.038
144. Kaplan S. Novikov l, Modan B. Nutritional factors in the etiology of brain tumors potential role of nitrosamines, fat, and cholesterol. *Am J Epidemiol.* (1997) 146:832–41. doi: 10.1093/oxfordjournals.aje.a009201
145. Lee H-W, Park S-H. Weng M-w, Wang H-T, Huang WC, Lepor H, et al. E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. *Proc Natl Acad Sci USA.* (2018) 115:E1560–9. doi: 10.1073/pnas.1718185115
146. Tanuja M. Ghutka and pr-cancerous lesions: An empirical study in 'twin city' of Odisha. *Int J Soc Econom Res.* (2018) 8:60–80. doi: 10.5958/2249-6270.2018.00011.9
147. Ramchandani AG, D'Souza AV, Borges AM, Bhisey RA. Evaluation of carcinogenic/co-carcinogenic activity of a common chewing product, pan masala, in mouse skin, stomach and esophagus. *Int J Cancer.* (1998) 75:225–32. doi: 10.1002/(SICI)1097-0215(19980119)75:2<225::AID-IJC10>3.0.CO;2-C
148. Nigam S, Kumar A, Sheikh S, Saiyed H. Toxicological evaluation of pan masala in pure inbred Swiss mice: a preliminary report on long-term exposure study. *Curr Sci.* (2001) 80:1306–9. Available online at: http://www.jstor.org/stable/24105045
149. Duncan RB, Briggs M. Treatment of uncomplicated anosmia by vitamin A. *Arch Otolaryngol.* (1962) 75:116–24. doi: 10.1001/archotol.1962.00740040122008
150. Christakos S, Li S, De La Cruz J, Bikle DD. New developments in our understanding of vitamin D metabolism, action and treatment. *Metabolism.* (2019) 98:112–20. doi: 10.1016/j.metabol.2019.06.010
151. Aguirre M, Manzano N, Salas Y, Angel M, Díaz-Couselo FA, Zylberman M. Vitamin D deficiency in patients admitted to the general ward with breast, lung, and colorectal cancer in Buenos Aires, Argentina. *Arch Osteoporos.* (2016) 11:4. doi: 10.1007/s11657-015-0256-x
152. Schloss J. Cancer treatment and nutritional deficiencies. In: Erkekoglu P, Kocer-Gumusel B, editors. *Nutritional Deficiency*. London: InTech (2016). p. 173–96. doi: 10.5772/63395
153. Lee E, Levine EA, Franco VI, Allen GO, Gong F, Zhang Y, et al. Combined genetic and nutritional risk models of triple negative breast cancer. *Nutr Cancer.* (2014) 66:955–63. doi: 10.1080/01635581.2014.932397
154. Xiao X, Wu Z-C, Chou K-C, A. multi-label classifier for predicting the subcellular localization of gram-negative bacterial proteins with both single and multiple sites. *PLoS ONE.* (2011) 6:e20592. doi: 10.1371/journal.pone.0020592
155. Adaramoye O, Akinloye O, Olatunji I. Trace elements and vitamin E status in Nigerian patients with prostate cancer. *Afr Health Sci.* (2010) 10:2.
156. Hurst R, Hooper L, Norat T, Lau R, Aune D, Greenwood DC, et al. Selenium and prostate cancer: systematic review and meta-analysis. *Am J Clin Nutr.* (2012) 96:111–22. doi: 10.3945/ajcn.111.033373
157. Deschasaux M, Souberbielle J-C, Latino-Martel P, Sutton A, Charnaux N, Druesne-Pecollo N, et al. Weight status and alcohol intake modify the association between vitamin D and breast cancer risk. *J Nutr.* (2016) 146:576–85. doi: 10.3945/jn.115.221481
158. Cho E, Zhang X, Townsend MK, Selhub J, Paul L, Rosner B, et al. Unmetabolized folic acid in prediagnostic plasma and the risk for colorectal cancer. *J Natl Cancer Inst.* (2015) 107:djv260. doi: 10.1093/jnci/djv260
159. Barrett CW, Singh K, Motley AK, Lintel MK, Matafonova E, Bradley AM, et al. Dietary selenium deficiency exacerbates DSS-induced epithelial injury and AOM/DSS-induced tumorigenesis. *PLoS ONE.* (2013) 8:e67845. doi: 10.1371/journal.pone.0067845
160. Steinfeld B, Scott J, Vilander G, Marx L, Quirk M, Lindberg J, et al. The role of lean process improvement in implementation of evidence-based practices in behavioral health care. *J Behav Health Serv Res.* (2015) 42:504–18. doi: 10.1007/s11414-013-9386-3
161. Lippi G, Mattiuzzi C, Cervellin G. Meat consumption and cancer risk: a critical review of published meta-analyses. *Crit Rev Oncol Hematol.* (2016) 97:1–14. doi: 10.1016/j.critrevonc.2015.11.008
162. Epplein M, Burk RF, Cai Q, Hargreaves MK, Blot WJ, A. prospective study of plasma Selenoprotein P and lung cancer risk among low-income adults. *Cancer Epidemiol Prevent Biomark.* (2014) 23:1238–44. doi: 10.1158/1055-9965.EPI-13-1308
163. Xue Y, Harris E, Wang W, Baybutt RC. Vitamin A depletion induced by cigarette smoke is associated with an increase in lung cancer-related markers in rats. *J Biomed Sci.* (2015) 22:1–9. doi: 10.1186/s12929-015-0189-0
164. Ho E, Courtemanche C, Ames BN. Zinc deficiency induces oxidative DNA damage and increases p53 expression in human lung fibroblasts. *J Nutr.* (2003) 133:2543–8. doi: 10.1093/jn/133.8.2543
165. Khan RM, Iqbal MP. Deficiency of vitamin C in South Asia. *Pakistan J Med Sci.* (2006) 22:347. Available online at: https://www.pjms.com.pk/issues/julsep06/article/review2.html
166. Carr AC, Rowe S. Factors affecting vitamin C status and prevalence of deficiency: a global health perspective. *Nutrients.* (2020) 12:1963. doi: 10.3390/nu12071963
167. White R, Nonis M, Pearson JF, Burgess E, Morrin HR, Pullar JM, et al. Low vitamin C status in patients with Cancer is associated with patient and tumor characteristics. *Nutrients.* (2020) 12:2338. doi: 10.3390/nu12082338

168. Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease. *Nutr Clin Care.* (2002) 5:66–74. doi: 10.1046/j.1523-5408.2002.00005.x
169. Lechner K, Obermeier HL. Cancer-related microangiopathic hemolytic anemia: clinical and laboratory features in 168 reported cases. *Medicine.* (2012) 91:195–205. doi: 10.1097/MD.0b013e3182603598
170. Clark SF. Iron deficiency anemia. *Nutr Clin Pract.* (2008) 23:128–41. doi: 10.1177/0884533608314536
171. Akhtar S, Ahmed A, Ahmad A, Ali Z, Riaz M, Ismail T. Iron status of the Pakistani population-current issues and strategies. *Asia Pac J Clin Nutr.* (2013) 22:340–7. Available online at: https://search.informit.org/doi/10.3316/ielapa.507285198519371
172. Nausheen S, Habib A, Bhura M, Rizvi A, Shaheen F, Begum K, et al. Impact evaluation of the efficacy of different doses of vitamin D supplementation during pregnancy on pregnancy and birth outcomes: a randomised, controlled, dose comparison trial in Pakistan. *BMJ Nutr Prevent Health.* (2021) 4:425. doi: 10.1136/bmjnph-2021-000304
173. Mustafa G, Asadi MA, Iqbal I, Bashir N. Low vitamin D status in nursing Pakistani mothers in an environment of ample sunshine: a cross-sectional study. *BMC Preg Childb.* (2018) 18:1–7. doi: 10.1186/s12884-018-2062-0
174. Moorani KN, Mustufa MA, Hasan SF, Kubar N. Vitamin D status in under five children in diverse communities of Karachi. *Pakistan J Med Sci.* (2019) 35:414. doi: 10.12669/pjms.35.2.680
175. Fund TUNICsE. *National Nutrition Survey 2018: Key Finding Report.* (2018). Available online at: https://www.unicef.org/pakistan/media/1871/file/KeyFindings---NationalNutritionSurvey2018.pdf
176. Griffin TP, Wall D, Blake L, Griffin DG, Robinson S, Bell M, et al. Higher risk of vitamin D insufficiency/deficiency for rural than urban dwellers. *J Steroid Biochem Mol Biol.* (2020) 197:105547. doi: 10.1016/j.jsbmb.2019.105547
177. Ali A. Current status of malnutrition and stunting in Pakistani children: what needs to be done? *J Am Coll Nutr.* (2021) 40:180–92. doi: 10.1080/07315724.2020.1750504
178. Siegel RL, Jakubowski CD, Fedewa SA, Davis A, Azad NS. Colorectal cancer in the young: epidemiology, prevention, management. *Am Soc Clin Oncol Educ Book.* (2020) 40:e75–88. doi: 10.1200/EDBK_279901
179. Reinke S, Taylor WR, Duda GN, Von Haehling S, Reinke P, Volk H-D, et al. Absolute and functional iron deficiency in professional athletes during training and recovery. *Int J Cardiol.* (2012) 156:186–91. doi: 10.1016/j.ijcard.2010.10.139
180. Saqib M, Manzoor F, Shahid R, Naz S, Sharif S. Incidence of anemia among pregnant females of rural areas of punjab. *S Asian J Life Sci.* (2019) 7:25–8. doi: 10.17582/journal.sajls/2019/7.2.25.28
181. Lowe NM, Zaman M, Moran VH, Ohly H, Sinclair J, Fatima S, et al. Biofortification of wheat with zinc for eliminating deficiency in Pakistan: study protocol for a cluster-randomised, double-blind, controlled effectiveness study (BIZIFED2). *BMJ Open.* (2020) 10:e039231. doi: 10.1136/bmjopen-2020-039231
182. Bhurgri Y. Karachi cancer registry data–implications for the national cancer control program of pakistan. *Asian Pac J Cancer Prev.* (2004) 5:77–82. Available online at: http://journal.waocp.org/article_24233.html
183. Ashraf MS, Jamil A. Cancer care in Pakistan. In: Silbermann M, editor. *Cancer Care in Countries and Societies in Transition*. Cham: Springer (2016). p. 231–45. doi: 10.1007/978-3-319-22912-6_15
184. Shamsi U. Cancer prevention and control in Pakistan: review of cancer epidemiology and challenges. *Liaquat Natl J Prim Care.* (2020) 2:34–8. Available online at: https://journals.lnh.edu.pk/lnjpc/pdf/7795eb42-d838-491d-99bb-aade6db21227.pdf
185. Wazir MS, Shaikh BT, Ahmed A. National program for family planning and primary health care Pakistan: a SWOT analysis. *Reprod Health.* (2013) 10:1–7. doi: 10.1186/1742-4755-10-60
186. World Health Organization. *Cancer Control: Knowledge Into Action: WHO Guide for Effective Programmes. Policy and Advocacy Module 6.* World Health Organization (2008).
187. Azhar S, Hassali MA, Ibrahim MIM, Ahmad M, Masood I, Shafie AA. The role of pharmacists in developing countries: the current scenario in Pakistan. *Hum Resour Health.* (2009) 7:1–6. doi: 10.1186/1478-4491-7-54
188. Ali A, Audi M. The impact of income inequality, environmental degradation and globalization on life expectancy in Pakistan: an empirical analysis (2016). doi: 10.14738/abr.511.3696. Available online at: https://mpra.ub.uni-muenchen.de/71112/
189. Nishtar S, Boerma T, Amjad S, Alam AY, Khalid F. ul Haq I, et al. Pakistan's health system: performance and prospects after the 18th Constitutional Amendment. *Lancet.* (2013) 381:2193–206. doi: 10.1016/S0140-6736(13)60019-7
190. Lee KS, Shahidullah A, Zaidi ST, Patel RP, Ming LC, Tariq MH, et al. The crux of the medicine prices' controversy in Pakistan. *Front Pharmacol.* (2017) 8:504. doi: 10.3389/fphar.2017.00504
191. Ahmad N. Status of higher education in nuclear technology in Pakistan (2007). Available online at: https://inis.iaea.org/search/search.aspx?orig_q=RN:40102297
192. Masood K, Ahmad M, Zafar J, ul Haq M, Ashfaq A, Zafar H. Assessment of occupational exposure among Pakistani medical staff during 2007-2011. *Austral Phys Eng Sci Med.* (2012) 35:297-300. doi: 10.1007/s13246-012-0156-y
193. Khurshid S. Nuclear medical centres of PAEC. *Nucleus.* (2020) 42:93–6. Available online at: http://thenucleuspak.org.pk/index.php/Nucleus/article/view/1051
194. Jamshed A, Syed AA, Shah MA, Jamshed S. Improving cancer care in Pakistan. *South Asian J Cancer.* (2013) 2:036–7. doi: 10.4103/2278-330X.105892
195. Pakistan CcharC. *Existing Cancer Burden & Treatment Facilities: Cancer Care Hospital and Research Centre Pakistan.* (2022). Available online at: https://cch-rc.com/why-cancer-hospital/ (accessed April 24, 2022).
196. Ashraf MS. Pediatric oncology in Pakistan. *J Pediatr Hematol Oncol.* (2012) 34:S23–5. doi: 10.1097/MPH.0b013e318249abf9
197. Iftikhar R, Mir MA, Moosajee M, Rashid K, Bokhari SW, Abbasi AN, et al. Diagnosis and management of diffuse large B-cell lymphoma: society of medical oncology, Pakistan society of hematology, and Pakistan society of clinical oncology Joint clinical practice guideline. *JCO Glob Oncol.* (2021) 7:1647–58. doi: 10.1200/GO.21.00320
198. Majeed FA, Azeem AR, Farhan N. Lung cancer in Pakistan, where do we stand. *JPMA.* (2019) 69:405–8. Available online at: https://www.jpma.org.pk/article-details/9083
199. HealthNet. *Cancer Care.* Available online at: http://healthnet.com.pk/cc.html (accessed March 23, 2022).
200. Sciences PIoM. *About PIMS.* Available online at: https://pims.gov.pk/aboutPIMS.htm (accessed March 28, 2022).
201. Tribune TE. *Free Food Facility Opened at PIMS.* (2022). Available online at: https://tribune.com.pk/story/2139614/free-food-facility-opened-pims (accessed April 01, 2022).
202. Oncology PSoC. *Mission and Objectives.* Available online at: https://psco.com.pk/about/#vision (accessed April 12, 2022).
203. Centre SKMCHaR. *About Us.* Available online at: https://shaukatkhanum.org.pk/about-us/
204. Pakistan SOS. *Surgical Oncology Society Pakistan.* Available online at: http://sospk.org/ (accessed April 26, 2022).
205. Biemar F, Foti M. Global progress against cancer-challenges and opportunities. *Cancer Biol Med.* (2013) 10:183–6. doi: 10.7497/j.issn.2095-3941.2013.04.001
206. Pierre-Louis AM, Akala FA, Karam HS. *Public Health in the Middle East and North Africa: Meeting the Challenges of the Twenty-First Century*. World Bank Publications (2004). doi: 10.1596/0-8213-5790-5
207. Naoum FA. Iron deficiency in cancer patients. *Rev Bras Hematol Hemoter.* (2016) 38:325–30. doi: 10.1016/j.bjhh.2016.05.009
208. Iniesta RR, Rush R, Paciarotti I, Rhatigan E, Brougham F, McKenzie J, et al. Systematic review and meta-analysis: prevalence and possible causes of vitamin D deficiency and insufficiency in pediatric cancer patients. *Clin Nutr.* (2016) 35:95–108. doi: 10.1016/j.clnu.2014.12.023

209. Gillberg L, Ørskov AD, Liu M, Harsløf LB, Jones PA, Grønbæk K (editors.). Vitamin C-A new player in regulation of the cancer epigenome. *Seminars Cancer Biol.* (2018) 51:59–67. doi: 10.1016/j.semcancer.2017.11.001
210. Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, et al. The role of vitamin D in cancer prevention. *Am J Publ Health.* (2006) 96:252–61. doi: 10.2105/AJPH.2004.045260
211. Marangoni F, Cetin I, Verduci E, Canzone G, Giovannini M, Scollo P, et al. Maternal diet and nutrient requirements in pregnancy and breastfeeding. An Italian consensus document. *Nutrients.* (2016) 8:629. doi: 10.3390/nu8100629
212. Stein AJ. Global impacts of human mineral malnutrition. *Plant Soil.* (2010) 335:133–54. doi: 10.1007/s11104-009-0228-2
213. Tippens G. Smokers: Nuisances in Belmont City, California-in their homes, but not on public sidewalks. *Minn JL Sci & Tech.* (2008) 10:413. Available online at: https://heinonline.org/HOL/LandingPage?handle=hein.journals/mipr10&div=15&id=&page=
214. Abbasi AS, Akhter W, Umar S. Ethical issues in advertising in Pakistan: an Islamic perspective. *World Appl Sci J.* (2011) 13:444–52. Available online at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=1866800
215. Wakefield M, Morley C, Horan JK, Cummings KM. The cigarette pack as image: new evidence from tobacco industry documents. *Tob Control.* (2002) 11:i73–80. doi: 10.1136/tc.11.suppl_1.i73
216. Gilani SI, Leon DA. Prevalence and sociodemographic determinants of tobacco use among adults in Pakistan: findings of a nationwide survey conducted in 2012. *Popul Health Metr.* (2013) 11:1–11. doi: 10.1186/1478-7954-11-16
217. Organization TCL. *The Cigarettes (Printing of Warning) Ordinance.* Available online at: https://www.tobaccocontrollaws.org/files/live/Pakistan/Pakistan%20%20-%201979%20Ordinance%20.pdf
218. Organization TCL. *Statutory Notifications Containing Rules and Orders issued by all Ministries and Divisions of the Government of Pakistan and their Attached and Subordinate Offices and the Supreme Court of Pakistan.* Available online at: https://www.tobaccocontrollaws.org/files/live/Pakistan/Pakistan%20-%20SRO%2086%28KE%29_2009%2C%2087%28KE%29_2009%20-%20national.pdf
219. Government of Pakistan Ministry of Finance EA, Statistics and Revenue. *Notifications (CUSTOMS).* Available online at: https://download1.fbr.gov.pk/SROs/2013102915104703 77SRO693(I)200629oct2013.pdf
220. Ministry of Health GoP. *Prohibition of Smoking and Protection of Non-Smokers Health Ordinance.* (2002). Available online at: http://www.tcc.gov.pk/Downloads/Prohibition%20of%20Smoking%20and%20Protection%20of%20Non-Smokers%20Ordinance%20%202002.pdf (accessed April 22, 2022).
221. Health GoPMo. *Notification.* Available online at: https://www.tobaccocontrollaws.org/files/live/Pakistan/Pakistan%20-%20SRO%20655%28I%29_2003%20-%20national.pdf (accessed May 01, 2022).

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The influence on fish and seafood consumption, and the attitudes and reasons for its consumption in the Croatian population

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Fish makes an important part of the Mediterranean diet, which has been scientifically proven to help preserve human health by protecting against major chronic and inflammatory diseases. Eating fish and seafood is very important, not only for its proven health benefits but also for its positive impact on the environment. Due to many fish and seafood significant positive effects on human health, this study aimed to investigate the socio-demographic factors associated with the consumption of fish and seafood in the population of Primorsko-goranska County in Croatia. Another aim was to determine people's attitudes, choices, and reasons for the consumption of fish and seafood. Self-reported data from 2,910 participants were used. According to the European dietary recommendations for fish consumption, the participants were divided into two groups; the very low to low fish consumption group and the moderate to high fish consumption group, in order to examine the differences in socio-demographic and lifestyle variables, and their attitudes, opinions, and reasons for fish and seafood consumption. More fish and seafood were consumed by women, the elderly, the more educated, non-smokers, and more physically active participants. Age, the highest level of education, and a diet even moderately adherent to the Mediterranean diet was found to significantly increase the likelihood of recommended fish consumption. Participants considered the best reasons to consume more fish lower prices, buy much more locally produced fishery products, and prefer to eat wild-caught fish rather than farmed fish. The study has found a slight increase in fish consumption, although still lower than the European average. It also showed significant socio-demographic associations, also the reasons and attitudes toward higher fish and seafood consumption of the Croatian population. The obtained research data are valuable for planning future public health programs in Croatia aimed at greater consumption of fish and seafood, as well as their promotion as an important part of a sustainable diet.

KEYWORDS

fish and seafood consumption, attitudes, choices, reasons, sustainable diet, consumers, Mediterranean diet (MD)

Introduction

Fish and seafood are foods of high nutritional value, rich in essential amino acids, high-quality proteins, many vitamins (A, B, and D) and minerals (iron, calcium, zinc, selenium), and especially omega-3 polyunsaturated fatty acids (Cardoso et al., 2013; Nesheim et al., 2015; Chen et al., 2022). Strong scientific evidence is confirming the beneficial effects of fish consumption on human health, including cognitive development, mental health, immune system, prevention of anemia, cardiovascular disease, and dementia (Béné et al., 2015; Golden et al., 2016). Fish consumption is an important part of the Mediterranean diet (MD), which has been scientifically proven to help preserve human health by protecting against major chronic and inflammatory diseases (Mazzocchi et al., 2019). In addition, there is a strong evidence suggesting a protective effect of the MD mostly on the risk of cardiovascular disease and certain types of cancer (Mazzocchi et al., 2019; Serra-Majem et al., 2019). The Mediterranean diet is not only considered as "the role model" of a healthy diet but also as a sustainable diet (Burlingame and Dernini, 2012; Portugal-Nunes et al., 2021) providing economic and socio-cultural benefits (Berry, 2019). However, regarding fish quality which is consumed as a part of the diet, its environmental effects can vary greatly between caught and farmed fish since the nutrient content depends on the fish's diet. The recommended weekly fish intake is often not reached due to insufficient supply (Berry, 2019).

Although the beneficial effects of MD on health are well known, the diet of many Mediterranean countries' residents is moving away from traditional MD. At the same time, those countries record a rise in overweight people, obesity, and chronic non-communicable diseases (FAO, 2015; Vilarnau et al., 2019).

As part of a healthy diet, it is usually recommended to consume at least two servings of different types of fish (∼240 g) each week, including one serving of oily fish. This consumption provides an average intake of 250 mg EPA + DHA, especially when fish replaces the consumption of less healthy foods (McGuire, 2016; Piepoli et al., 2016). The consumption of fish and seafood varies in all European countries, although according to the Food and Agriculture Organization (FAO), consumption of fish and seafood has increased over the last 60 years (FAO, 2020). A trend toward increasing consumption of fish and seafood can also be observed in Croatia. In 2000, per capita consumption was 7.26 kg and in 2013 it was 19.06 kg, which is an increase of 162.5% (EUMOFA, 2019). The average consumption of fish and seafood in 2017 in the European Union was 24.35 kg per capita, whereas, in Croatia, the consumption in 2017 was 18.7 kg per capita (EUMOFA, 2019). Although the consumption increased by 6% (19.19 kg on average) in 2018, it still remained below the European average consumption (EUMOFA, 2020).

Various factors, such as place of residence (coast or continent), socio-demographic factors, tradition, habits, age, gender, etc. have been shown to influence seafood consumption (Almeida et al., 2015; Murray et al., 2017; Govzman et al., 2021). Moreover, fish and seafood consumption is also influenced by modern lifestyles, urbanization, and consumer preferences (Crona et al., 2020) likewise consumer attitudes toward food and nutrition all have been shown to be important factors influencing seafood consumption (Hearty et al., 2007).

Some of the main factors of insufficient consumption of fish and seafood include price or preferences, but it is worth mentioning that consumer habits and lifestyle can be influenced in the long term (Ministarstvo poljoprivrede, Uprava ribarstva, oŽujak, 2021). Public health activities should be undertaken to influence changes in consumers eating habits toward increasing the consumption of fish and seafood. This paper aims to study the socio-demographic factors related to the consumption of fish and seafood among the population of the Primorsko-goranska County in Croatia. Another aim is to determine people's attitudes, choices, and reasons for the consumption of fish and seafood.

Materials and methods

Participants

This is a quantitative cross-sectional study conducted in the population of Primorsko-Goranska County, Croatia. The study has been carried out under the project "Population exposure to traditional and emerging contaminants due to the consumption

TABLE 1 Socio-demographic characteristics and mean consumption of fish and fishery products among Croatian adults from Primorsko-goranska County ($N = 2,910$).

	N (%)	*p*-value*	Mean (g/week)	SD	*p*-value**
Gender					
Male	427 (14.7)	<0.001	406.24	349.76	0.363
Female	2,483 (85.3)		412.79	367.90	
Age group (years)					
20–30	328 (11.3)	<0.001	457.04	419.19	<0.001
31–40	1,717 (59.0)		392.14	345.03	
41–50	698 (24.0)		409.68	346.85	
51 +	167 (5.7)		526.98	465.50	
Highest level of education					
Postgraduate degree/Graduate diploma/Bachelor degree	1,731 (59.5)	<0.001	424.55	378.29	0.013
High school/Elementary school diploma	1,179 (40.5)		393.95	346.93	
Employment status					
Employed	2,489 (85.5)	<0.001	415.88	374.63	0.328
Unemployed	402 (13.8)		381.24	296.88	
Retired	19 (0.7)		391.32	166.62	
Income (Croatian average salary)					
≤1–2	1,661 (57.1)	<0.001	412.26	372.51	0.630
>2–3	671 (23.1)		399.72	356.12	
>3	271 (9.3)		434.86	324.54	
Don't want to respond	307 (10.5)		415.57	399.57	
Physical activity level					
Low	561 (19.3)	<0.001	409.30	388.77	0.049
Moderate	1,720 (59.1)		401.86	358.51	
High	629 (21.6)		443.48	359.59	
Smoking habits					
Non-smoker	1,965 (67.5)	<0.001	400.96	347.20	0.025
Smoker	945 (32.5)		430.13	381.61	
Nutrition status					
Underweight	81 (2.8)	<0.001	437.07	364.91	0.887
Normal weight	1,984 (68.2)		411.75	364.48	
Overweight	797 (27.4)		414.64	398.23	
Obese	48 (1.7)		442.73	305.19	
Mediterranean diet score (points)					
Not adherent (≤5)	1,132 (38.9)	<0.001	357.60	344.02	<0.001
Moderately adherent (6–9)	1,661 (57.1)		430.76	358.27	
Adherent (≥10)	117 (4.0)		679.40	494.63	

*Chi squared test (categorical variables).
**One-way ANOVA test (continuous variables).

of seafood and the characterization of health risks". The study was conducted in the period from October 2019 to February 2020 and approved by the Ethical Committee of the Teaching Institute of Public Health of Primorsko-Goranska County. Based on a number of residents of Primorsko-goranska county ($N = 296,195$) (Health Statistical Yearbook of Primorsko-Goranska County for 2020, 2022) a total of 7,745 questionnaires were sent to the residents older than 18 years of Primorsko-goranska County, out of which 5,542 (71.6%) have been returned. For the purposes of this study, only those questionnaires that were completed were further analyzed. Therefore, this study included questionnaires of 2,910 participants (37.6%).

The questionnaire

The questionnaire was anonymous and consisted of three sections. The first section included data on fish and seafood consumption (type of fish and seafood consumed), general

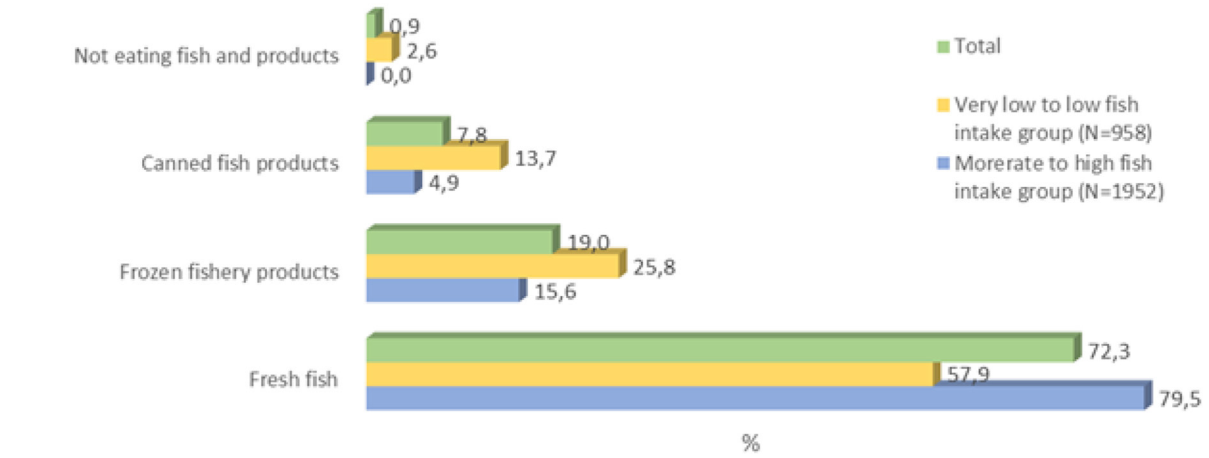

FIGURE 1
The proportion of fishery products consumption among groups regarding recommended fish intake ($N = 2,910$).

habits, preferences and frequency of buying fish, locations and reasons of buying, reasons for consumption, attitudes, and opinions about fish preferences based on its origin (i.e., marine and farmed), and about sources of information on the positive health effects of fish consumption. Participants graded the statements about farmed fish using the Likert scale with five ratings ranging from "strongly disagree" (1) to "strongly agree" (5). The second part was related to sociodemographic characteristics and lifestyle of the study participants and included data on age, gender, geographical region, education level, income, occupation, body weight and height, physical activity, and smoking habits. The third part of the questionnaire evaluated dietary habits. Participants noted their habitual fish intake in offered intervals from once per month or less than to every day, separately for eating at home and for eating in restaurants and/or at work. They also noted their average fish consumption portion in kilograms per household member (estimate based on the mean value of portion consumption and number of household members). Since the Primorsko-goranska County resides by the Mediterranean Sea where the traditional Mediterranean diet is a hereditary diet, the adherence to the MD was assessed with the 14-Item Mediterranean Diet Assessment Tool (Martínez-González et al., 2012). The adherence to MD has categorized into three levels; $\leq$5 points which meant low adherence, from 6 to 9 points which meant moderate adherence and $\geq$10 points which meant high adherence to MD. To explore differences in attitudes, opinions, and reasons toward fish and seafood consumption regarding habits of fish consumption, the participants were divided into two groups: the very low to low fish intake group (VLFI; i.e., <1 serving/month to 1 serving/week) and to moderate to high fish intake group (MHFI; i.e., 2–5 servings/week), based on the European dietary recommendations for fish consumption (Lofstedt et al., 2021), where one serving of fish is 130 g.

Statistical analysis

To describe habits of fish and seafood consumption among sociodemographic subgroups of participants, the proportion and means of fish and seafood consumption were calculated and tested for differences with one-way ANOVA test for continuous variables, or with the Chi-Square test for sociodemographic subgroups. The Chi-square test was used for differences between subgroups of participants based on their habitual fish consumption regarding recommended fish intake (Lofstedt et al., 2021) for their attitudes, choices and reasons for consuming fish and seafood and farmed fish. The quantification of the influence of the individual factors is expressed by the odds ratio calculated with the logistic regression method. All tests were performed using Statistica 12.7 for Windows (Statsoft Inc., Tulsa, OK, USA). Results with a P-value of <0.05 were considered statistically significant.

Results

Socio-demographic characteristic and consumption of fish and seafood

The study consisted of 2,910 residents of the Primorsko-goranska County older than 18 years of which over four-fifths (85.3%) were females ($p < 0.001$; Table 1). Their

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average age was 38.00 ± 7.14 years, and the most of participants were in the 31–40 years age group (59.0%, $p < 0.001$). There were statistically more participants with higher education ($p < 0.001$), employed ($p < 0.001$), with two average Croatian salaries ($p < 0.001$), moderately physically active ($p < 0.001$), non-smokers ($p < 0.001$), with normal weight ($p < 0.001$) and diet that moderately adhered to the Mediterranean diet ($p < 0.001$). On average, participants' fish and fisheries product consumption per week was 412.28 ± 365.01 g (data not shown). Mostly consumed was fresh fish (72.3%), followed by frozen fisheries products (19.0%) and canned fish products (7.8%) (Figure 1). When choosing their most consumed types of fish and fisheries products, participants consumed mainly white fish (56.5%), followed by cephalopods (52.3%), fatty fish (43.9%), and crabs (27.1%) (Figure 2). In Table 1 there are presented the participants' mean fish intake according to their characteristics. Participants in the 51+ age group ate statistically significantly more fish than other age groups ($p < 0.001$). Participants with higher education ($p = 0.013$), those who were highly physically active ($p = 0.049$), smokers ($p = 0.025$) and those with high adherence to MD ($p < 0.001$) ate statistically more fish than their related groups. Average weekly consumption of fish didn't significantly differ according to employment, income, and nutrition status (Table 1).

Based on European dietary recommendations for fish consumption (Lofstedt et al., 2021), the moderate to high fish consumption group (MHFI) consumed statistically 2.5 more fish per week (511.12 ± 410.77 g) than the very low to low fish consumption group (VLFI) group (206.05 ± 172.95 g, $p < 0.001$) (data not shown). The MHFI group consumed statistically significantly more fresh fish than the VLFI group (79.5 vs. 57.9%, $p < 0.001$). The VLFI group had significantly higher consumption of frozen fisheries products (28.8 vs. 15.6%, $p < 0.013$) and canned fish (13.7 vs. 4.9%, $p < 0.020$) than the MHFI group (Figure 1).

Regarding the fish and fishery products, the MHFI group consumed significantly more fatty fish (47.6%), crabs (30.0%), cephalopods (54.7%), and molluscs (9.7%) than the VLFI group (36.4, 21.2, 47.3, and 5.5%, $p < 0.001$). The VLFI group consumed significantly more white fish and freshwater fish than MHFI group (62.4 vs. 60.9%, $p < 0.001$; 9.1 vs. 8.6%, $p < 0.001$) (Figure 2).

Socio-demographic and lifestyle factors influencing consumption of fish and seafood

The socio-demographic and lifestyle factors influencing recommended fish and fishery product consumption were assessed by logistic regression (Table 2). It was shown that age was an important influencing factor, the probability of recommended fish intake significantly increases by 1.38 times in older than 40 years, or about 40% ($p = 0.001$), while among younger people that probability reduces by 18% ($p = 0.035$) to 35% ($p = 0.001$). The highest education level significantly increases that probability by 1.72 times, or about 70% ($p = 0.001$) while the lower one reduces the probability by 19% ($p = 0.033$).

Non-smoking and smoking a smaller number of cigarettes had no statistically significant effect while smoking a larger number of cigarettes reduces the probability by 1.43 times ($p = 0.004$). Also, having a normal weight significantly increases the probability by 1.24 times ($p = 0.008$) while having obesity significantly reduces that chance by 1.32 times ($p = 0.025$). So, if the person smokes more or has obesity,

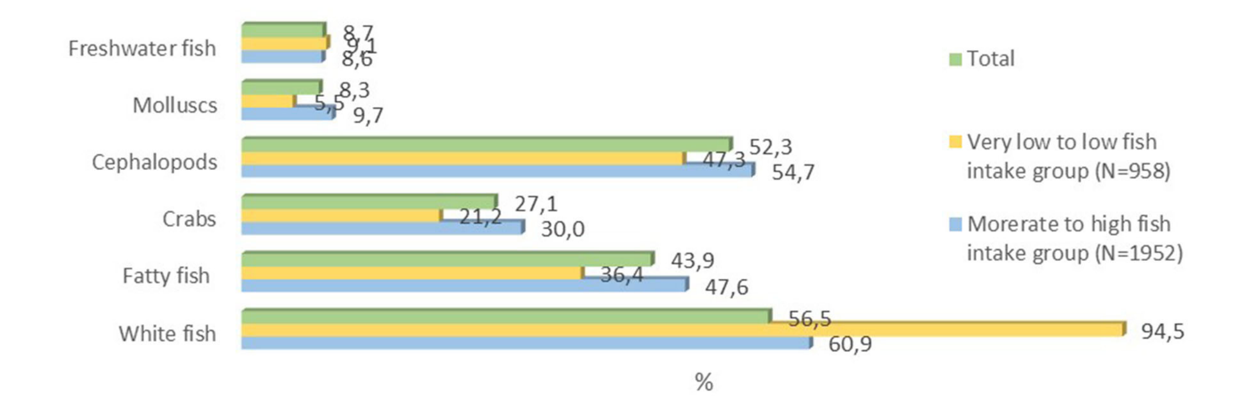

FIGURE 2
Fishery products frequency consumption among groups regarding recommended fish intake ($N = 2,910$).

TABLE 2 The association of participants' socio-demographic characteristics and lifestyle habits with recommended fish intake.

Variable	OR	95% CI	*P*-value
Age group			
<30 years	0.74	0.58–0.94	0.014
31–40 years	0.85	0.72–0.99	0.035
41–50+ years	1.38	1.17–1.63	0.001
Highest level of education			
Elementary school	1.18	0.70–2.00	0.538
High school	0.84	0.72–0.99	0.033
Graduated/Bachelor degree	0.99	0.85–1.16	0.945
Postgraduate degree	1.72	1.27–2.34	0.001
Smoking habits			
No	1.16	0.99–1.37	0.071
1–10 cigarettes	1.11	0.91–1.35	0.320
11–20 cigarettes	0.70	0.55–0.89	0.004
Physical activity level			
Low	1.11	0.92–1.34	0.284
Moderate	0.94	0.81–1.10	0.471
High	0.98	0.80–1.19	0.827
Nutrition status			
Underweight	1.19	0.72–1.96	0.507
Normal weight	1.24	1.06–1.45	0.008
Overweight	0.86	0.72–1.03	0.109
Obese	0.76	0.60–0.97	0.025
Mediterranean diet score			
Not adherent	0.49	0.42–0.58	<0.001
Moderately adherent	1.66	1.42–1.95	<0.001
Adherent	5.43	2.83–10.44	<0.001

the likelihood to consume fish as recommended decreases by about 30–40%.

On the other hand, a diet even moderate adherent to the Mediterranean diet compared to a diet that is not adherent to MD significantly increases the probability of recommended fish intake by 1.66 times, or about 70% ($p < 0.001$) while a diet that is completely adherent MD increases that probability five times ($p < 0.001$).

The participants also reported the most important information available on fishery products (Figure 3). Expiration dates were the most important information about fishery products for participants in both groups, slightly more important for the VLFI group (44.5% MHFI and 48.4% VLFI, respectively, $p = 0.046$) as well as fishing area or country of origin (21.6% MHFI and 19.3% VLFI, respectively, $p = 0.150$). Whether fishery products were previously frozen was significantly more important for the MHFI group than for the VLFI group (13.5 and 10.4%, respectively, $p = 0.016$).

Attitudes, choices, and reasons for the consumption of fish and seafood

In Table 3 are presented reasons for encouraging to consume more fish and fishery products according to recommended fish intake group. In both groups, participants considered that the best reasons for promoting the higher fish consumption were lower prices (56.8 and 56.2%, respectively, $p = 0.758$) and better availability in stores (47.2 and 44.2%, respectively, $p = 0.117$). With a significant difference, participants in the MHFI group placed more importance on highlighted labels of quality or origin (31.1 and 25.3%, respectively, $p = 0.001$), while participants in the VLFI group provided more the information about fish preparation recipes (14.5 and 9.2%, respectively, $p < 0.001$). Other reasons weren't statistically different according to recommended fish intake.

When choosing their main reasons for consumption of fish and fishery products, participants in the MHFI group chosen as the most important reasons the taste (69.5 vs. 58.4%, $p < 0.001$), health consideration (84.6 vs. 72.3%, $p < 0.001$), availability (9.7 vs. 5.3%, $p < 0.001$), good quality (15.1 vs. 5.1, $p < 0.001$) and a habit to eat (42.4 vs. 18.8%, $p < 0.001$) significantly more than participants in the VLFI group (Figure 4). Participants were asked to declare from which information sources they learn about the importance of consuming fishery products. All declared that they the most got the information from their friends, relatives and families, significantly more the MHFI group (60.2% of the MHFI and 51.3% of the VLFI, $p < 0.001$), while the VLFI group significantly more stated to get it online (47.8 MHFI and 51.3 VLFI, $p = 0.080$). Only a fifth of participants (22.7% of MHFI and 19.5% of VLFI, $p = 0.051$) declared that information has been provided by health workers, including physicians, nutritionists, etc. (Figure 5).

Regarding the choices and reasons for purchasing fishery products by its origin, the MHFI group was statistically significantly more likely to purchase fishery products from Croatia, i.e., locally (49.1, 39.7%, respectively, $p < 0.001$) or from all Croatia regions (40.1, 36.0%, respectively, $p = 0.035$) when compared to the VLFI group. Significantly 2.5 times more the VLFI group said that the origin doesn't matter to them and/or didn't know the origin than the MHFI group ($p < 0.001$; Table 3). More precisely, the main reasons of buying the fishery product by its origin for both studied groups were first of all better quality and taste (58.7 and 42.3%, respectively, $p < 0.001$), then availability (41.1 and 39.5%, respectively, $p = 0.400$), habit (29.3 and 26.0%, respectively, $p < 0.062$), and support to local fishermen, farmers and for job preservation reasons (28.3 and 19.4%, respectively, $p < 0.001$) (Table 4).

Regarding the wild caught or farmed fish consumption preference, both studied groups of participants preferred more

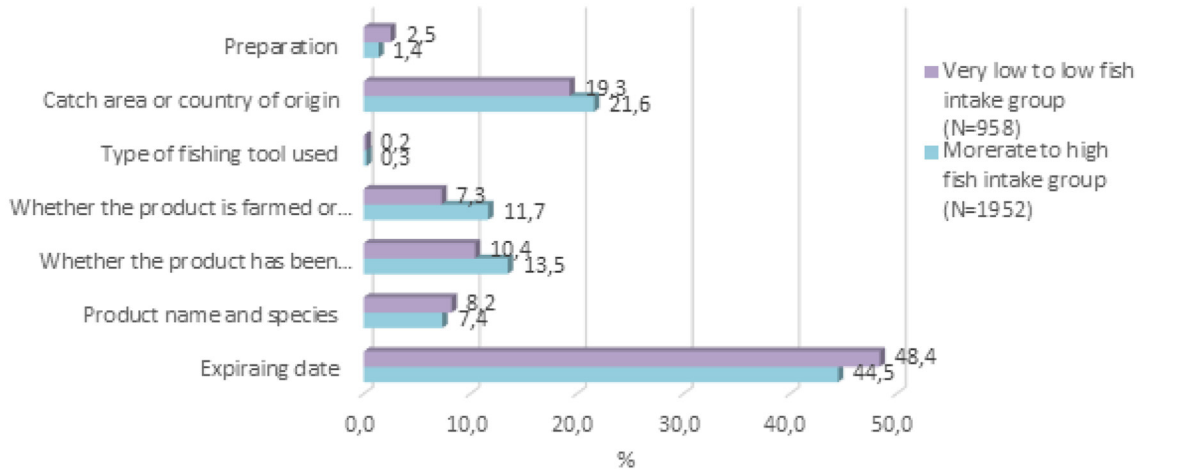

FIGURE 3
The most relevant information on fishery products among groups regarding recommended fish intake.

TABLE 3 Reasons for encouraging to consume more fish and fishery products among groups regarding the recommended fish intake ($N = 2,910$).

	MHFI ($N = 1,952$)	VLFI ($N = 958$)	p-value*
Better availability in stores	922 (47.2)	423 (44.2)	0.117
Better availability in restaurants	36 (1.8)	23 (2.4)	0.317
Lower prices	1,108 (56.8)	538 (56.2)	0.758
Better availability of information on the impact of fish diet on health	155 (7.9)	73 (7.6)	0.763
Highlighted marks of quality or origin	607 (31.1)	242 (25.3)	0.001
More information on recipes to prepare fish	180 (9.2)	139 (14.5)	<0.001
Introduction of special educational courses on preparing fish for eating	66 (3.4)	46 (4.8)	0.061
Nothing/I don't need to consume it more often	299 (15.3)	139 (14.5)	0.567

MHFI, moderate to high fish intake group; VLFI, very low to low fish intake group.
*Chi square test between the observed groups.

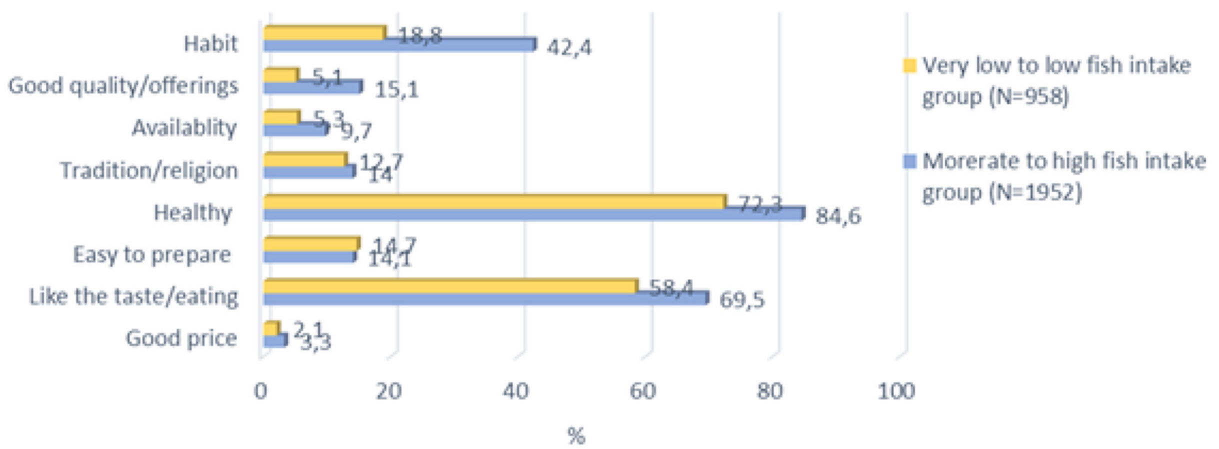

FIGURE 4
Reasons to consume fish and fishery products among groups regarding the recommended fish intake.

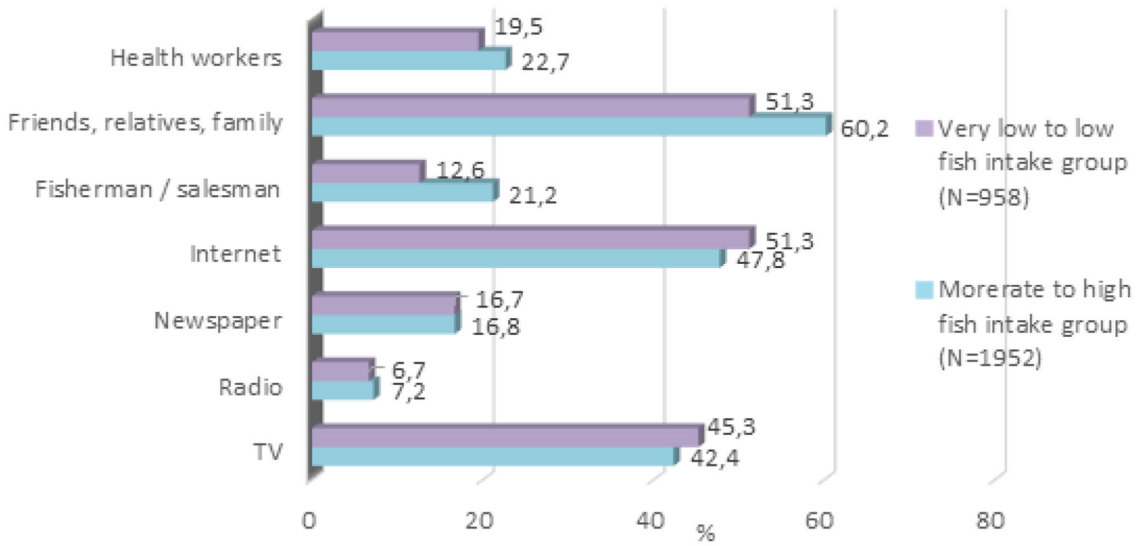

FIGURE 5
All information channels for knowing the importance of consuming fishery products among groups regarding recommended fish intake.

TABLE 4 Choices and reasons to buy fishery products by its origin ($N = 2,910$) among groups regarding the recommended fish intake.

	MHFI ($N = 1,952$)	VLFI ($N = 9\ 58$)	p-value*
The most common origin of bought fishery products			
Croatia—local region	959 (49.1)	380 (39.7)	<0.001
Croatia—all regions	782 (40.1)	345 (36.0)	0.035
Imports from EU countries	84 (4.3)	61 (6.4)	0.016
Imports from non-EU countries	15 (0.8)	12 (1.3)	0.200
It doesn't matter to me/I don't know the origin	113 (5.8)	136 (14.2)	<0.001
Reasons to make that choice of buying the fishery product by its origin			
Better price	128 (6.6)	83 (8.7)	0.039
Better quality and taste	1,146 (58.7)	405 (42.3)	<0.001
A better relationship between price and what I get for that price	269 (13.8)	132 (13.8)	<0.001
Better choice/greater products offer	290 (14.9)	80 (8.4)	<0.001
Availability	802 (41.1)	378 (39.5)	0.400
Habit/previous experience	572 (29.3)	249 (26.0)	0.062
Support to local fishermen/farmers/ob preservation	552 (28.3)	186 (19.4)	<0.001

MHFI, moderate to high fish intake group; VLFI, very low to low fish intake group.
*Chi square test between MHFI and VLFI group.

eating a wild caught fish over a farmed fish, significantly more the MHFI group than the VLFI group (68.4 and 51.6%, respectively, $p < 0.001$) (data not shown).

Table 5 shows the respondents' attitudes toward farmed fish and its quality. The MHFI group mostly agreed that farmed fish are fatter than wild fish and of a poorer quality ($p < 0.001$), while the VLFI group was mostly uncertain about that ($p < 0.001$; $p < 0.001$). Both groups mostly were uncertain about that the farmed fish is too expensive ($p < 0.001$). The MHFI group the group was undivided about the knowledge about farmed fish, in almost equal shares the participants agreed that they know and that they do not know and that they agree and disagree, while the VLFI group significant mostly agreed that they don't know enough about farmed fish ($p < 0.001$).

Discussion

This study is one of the very few conducted in Croatia that investigated factors influencing fish and seafood consumption

TABLE 5 Attitudes toward farmed fish and its quality ($N = 2,910$) among groups according to recommended fish intake.

Statement	I disagree	I neither agree nor disagree	I agree	*p*-value*	*p*-value**
Farmed fish is fatter than wild					
MHFI ($N = 1,952$)	378 (19.4)	692 (35.5)	882 (45.2)	<0.001	<0.001
VLFI ($N = 958$)	200 (20.9)	398 (41.5)	360 (37.6)	<0.001	
Farmed fish is of poorer quality than wild					
MHFI	437 (22.4)	712 (36.5)	803 (41.1)	<0.001	<0.001
VLFI	250 (26.1)	382 (39.9)	326 (34.0)	<0.001	
Farmed fish has an unnatural shape, color, and taste					
MHFI	863 (44.2)	683 (35.0)	406 (20.8)	<0.001	0.119
VLFI	452 (47.2)	335 (35.0)	171 (17.8)	<0.001	
Farmed fish is too expensive					
MHFI	732 (37.5)	760 (38.9)	460 (23.6)	<0.001	0.041
VLFI	317 (33.1)	414 (43.2)	227 (23.7)	<0.001	
I don't know enough about farmed fish					
MHFI	651 (33.4)	668 (34.2)	633 (32.4)	0.641	<0.001
VLFI	264 (27.6)	296 (30.9)	398 (41.5)	<0.001	

MHFI, moderate to high fish intake group; VLFI, very low to low fish intake group.
*Chi square test within a particular group (MHFI and VLFI group).
**Chi square test between MHFI and VLFI group.

and examined the reasons for their consumption. Regarding available similar studies (Tomić et al., 2016; Thong and Solgaard, 2017; Sacchettini et al., 2021), this study also assessed the average fish intake regarding socio-demographic characteristics of participants, and the proportion of particular seafood in the coastal part of Croatia regarding recommended fish intake (Lofstedt et al., 2021).

Fish and seafood consumption of croatian participants

Although there's a trend of increasing consumption of fish and seafood in Croatia (EUMOFA, 2019), it has remained below the European average consumption (EUMOFA, 2020), and this study confirmed that. On average, study participants consumed fish and aquatic products 13% less than the EU weekly average and 12% more than the Croatian (EUMOFA, 2020). Those participants who consumed fish two to five servings per week consumed 28% more than the Croatian average consumption, while those who consumed fish less than one serving per month to one serving per week consumed slightly more than half of the Croatian average consumption (EUMOFA, 2020). Of all aquatic food, participants mostly consumed fresh fish, preferably white over fatty fish. These results are consistent with consumption in Portugal and Spain, which also reported the highest consumption of fresh fish, followed by frozen and canned fish (Cardoso et al., 2010, 2013; MARM, 2010). Interestingly, cephalopods have been consumed more than fatty fish which is consistent with the results from Norway (Trondsen et al., 2003), and opposite to Portugal, where more fatty fish was consumed (Cardoso et al., 2013). In Norway salmon, as the most common fatty fish, is traditionally an expensive food, so, understandably, more white fish is eaten there. In Croatia, on the other hand, anchovy and sardines, as the most common fatty fish, are cheaper than white fish, so the data that participants ate less fatty than white fish is worrying, considering that fatty fish over white have as beneficial content of omega—3 fatty acids (McManus and Merga, 2011; Abdelhamid et al., 2018). Cephalopods were slightly more consumed than fatty fish. Compared with sardines and salmon, cephalopods such as squid, octopus, and cuttlefish contain lower energy due to lower content of total fat, and omega—3 fatty acids, but contain a higher quantity of cholesterol, vitamin A and most of the minerals, especially calcium (Food data, 2019).

An increasing interest is arising for more diverse and sustainable use of cephalopods, not only for culinary, but for novel uses to compensate for the declining fisheries of bonefish, and to identify them as additional protein sources to replace meat from land-animal production (Mouritsen and Styrbæk, 2018). In addition, it was shown that cephalopod species may be classified as potentially healthy food due to their ideal atherogenic/thrombogenic and hypocholesterolemic/hypercholesterolemic ratios based on fatty acid/amino acid ratios (Chakraborty et al., 2016). Although many studies have confirmed the beneficial effects of fish consumption on cardiovascular health, mainly due to the content of omega-3 fatty acids, also high-quality proteins, vitamins, and minerals (de Roos et al., 2017; Abdelhamid et al., 2018; Chen et al., 2022), recently it was concluded that fish consumption possesses also antioxidative, anti-inflammatory, immune-protective, wound healing, neuroprotective, cardioprotective, and hepatoprotective properties, not only for the content of omega-3 fatty acids but for substantial content of vitamin D, proteins, such as immunoglobins and amino-acids such as arginine (Chen et al., 2022). This study found that two-thirds of participants consumed fish and seafood two or more times a week, and three-fifths of participants had a diet that moderately and highly adhered to the Mediterranean diet, which represents important information and a prospective beneficial foundation for good health. The results showing a substantial proportion of participants with a diet moderately to highly adhering to MD is affirmative, since previous Croatian studies showed the MD diminishing, especially among younger populations (Kolčić et al., 2016; Kendel Jovanović et al., 2020; Šarac et al., 2021). Despite the well-known health benefits of fish and seafood consumption, mainly due to the content of omega-3 fatty acids, there is a potential health risk of fish and seafood consumption. Recent studies have pointed to them as a potential dietary source of exposure to various environmental pollutants with well-known potential adverse effects on human health (Domingo, 2016; Vilavert et al., 2017). The most pronounced contaminants investigated in fish and seafood are methylmercury and polychlorinated biphenyls, heavy metals and organohalogenated compounds, and also polycyclic aromatic hydrocarbons. It was shown that the exposure to the abovementioned environmental contaminants can be managed by the frequency and amount of consumption as well as controlled consumption of particular seafood species, such as predatory fish regarding their potential risk for pregnant and nursing women and also children (EFSA, 2012). Regarding this potential health risk, it is worthy to examine the potential health risk for Croatian fish and seafood consumers assessed according to the average consumption of various species of fish and seafood provided by this study.

Factors influencing fish and seafood consumption

Regarding fish and seafood consumption, many factors affect its consumption such as place of residence (coast or continent), socio-demographic factors, tradition, habits, age, gender, and many others (Murray et al., 2017; Govzman et al., 2021; Sacchettini et al., 2021). The study results confirmed that older age, higher education level, non-smoking, normal weight and adherence to the Mediterranean diet were significant and strongest determinants for higher fish and seafood consumption. It was shown that age is one of the most important factors influencing fish and seafood consumption (Akbaraly and Brunner, 2008; Jahns et al., 2014; Marushka et al., 2018). Study results are also consistent with the findings of studies showing a positive association between older age and fish consumption (Akbaraly and Brunner, 2008; Buscemi et al., 2014; Jahns et al., 2014). A small number of studies noted a positive association between fish consumption and younger people (Mozaffarian et al., 2008; van Woudenbergh et al., 2009; Anderson et al., 2010; Virtanen et al., 2010; Belin et al., 2011; Bonaccio et al., 2017), which represents an important public health task for promoting fish consumption among the younger population. A previously established positive association between educational level and fish and seafood consumption (van Woudenbergh et al., 2009; Anderson et al., 2010; Levitan et al., 2010; Heppe et al., 2011; Larsson et al., 2011; Giuli et al., 2012; Nordgren et al., 2017) was also confirmed in this study. Non-smoking presented to be positively associated with fish consumption (He et al., 2009; van Woudenbergh et al., 2009; Sala-Vila et al., 2011; Giuli et al., 2012; Langlois and Ratnayake, 2015), and this study found that smokers were significantly less likely to consume fish compared to non-smokers. No clear association between body weight and fish and seafood consumption was shown in this study, although participants with obesity were significantly less likely to consume fish and seafood than other sub-groups. Mixed results are available about this association, some studies have shown a positive association (He et al., 2009; Hostenkamp and Sørensen, 2010; Belin et al., 2011; Heppe et al., 2011; Belle et al., 2017) and others did not (van Woudenbergh et al., 2009; Meier et al., 2010; Kossioni and Bellou, 2011; Nesheim et al., 2015; Wallin et al., 2017). The results of this study showed that a diet that is even moderately adherent to the Mediterranean diet significantly increases the probability of recommended fish intake while high adherence increases that probability by five-fold. Fish and seafood, recognized as foods of high nutritional value (Cardoso et al., 2013; Nesheim et al., 2015; Chen et al., 2022), are represented as an important part of the Mediterranean diet, highly abundant in plant-based foods, which are considered as a model for a healthy and sustainable diet (Burlingame and Dernini, 2012; Portugal-Nunes et al., 2021). Dietary guidelines for sustainable diets generally recommend diets that are based on plant-based foods, because the majority of them tend to have some of the lowest environmental impacts given their nutrient content (Willett et al., 2019). Still, some fish, such as small pelagic fishes, were shown to be among the most healthful and sustainable foods (Springmann et al., 2020; Koehn et al., 2022), likewise some wild-caught fisheries and farmed shellfish, which showed to have low environmental impacts similar to many plant-based foods, and lower than most animal-source proteins (Koehn et al., 2022). Therefore, by including more fish in a diet, and making it more adherent to the MD, a diet that is proven to sustain human health by protecting it against the main chronic and inflammatory diseases (Mazzocchi et al., 2019), represents a significant step toward a more sustainable and healthier diet and way of living. Therefore, promoting a Mediterranean diet style with sustainable fish consumption through education is a very important way to transfer knowledge to every population group, especially the younger ones. Also, teaching families could be of greater importance because parenteral dietary habits have a strong influence on children's eating habits (Sotos-Prieto et al., 2015).

When choosing fish, for this study participants the most important information about fishery products was the expiration date of the country of origin. More frequent fish and seafood consumers were significantly more likely to buy fish and seafood from Croatia, i.e., locally, while those less frequent fish and seafood consumers indicated the origin as less important. It can be supposed that participants who more frequently consumed fish and seafood believed that fish and seafood from Croatia have better quality and taste, is more accessible, and when buying, they did it as a support local fishermen and to help the local economy. Those participants also consumed significantly more fatty fish than those who were less frequent consumers, which is supported by the information that more frequent consumers chose health as the most important reason for fish consumption, which is in line with other studies (Govzman et al., 2021; Sacchettini et al., 2021).

The frequency of consuming fish within recommendation didn't influence the consumption of wild-caught or farmed fish, since both study groups consumed more wild-caught fish than farmed fish, which is similar to results obtained in Portugal (Cardoso et al., 2013) and other European countries (Verbeke et al., 2005). The consumption of more wild-caught fish by study participants is consistent with their attitudes toward farmed fish and its quality. More frequent fish and seafood consumers significantly agreed that farmed fish is fatter than wild-caught, and thus has poorer quality. They reported farmed fish to have an unnatural shape, color, and taste. Participants who were less frequent consumers were significantly uncertain about that issue. Those results represent an area that should be further examined for a better insight on the results.

This study looked at what measures would be the most appropriate to promote the consumption of fish and seafood. Participants from both groups agreed that this would be a lower price and better availability in stores. Many studies also mention that the price of fish is a barrier to its consumption (Altintzoglou et al., 2010; Grieger et al., 2012; Lawley et al., 2012; Dijkstra et al., 2014; Bishop and Leblanc, 2017). Sotos-Prieto et al. in their study mentioned that people with lower income eat less fish, and although in this study the participants with higher income ate slightly more fish, this difference was not significant (Sotos-Prieto et al., 2015). As the main reason for consuming fish and seafood, more frequent fish and seafood consumers from this study significantly chose taste and health. Many studies confirmed that the taste of fish influenced its higher consumption (Sveinsdottir et al., 2009; Appleton, 2016; Bostic et al., 2017; Christenson et al., 2017). The belief that fish contains nutrients that have a positive effect on health also leads to higher fish and seafood consumption (Altintzoglou et al., 2010; Birch et al., 2012; Rahmawaty et al., 2013; Jacobs et al., 2018), which this study also confirmed. Interestingly, both groups in this study reported that they get the most information about the importance of fish consumption from friends, relatives, family, then the Internet and TV, and only a fifth from health workers. Rahmawaty et al. (2013) mentioned in their study that those family members who often do not eat fish negatively influence the fish consumption of other family members. This study showed that the frequency of consuming fish and seafood products wasn't related to the source of information about the health benefits of consuming fish, but it is suggesting that the role of health workers should be stronger in promoting the health benefits of fish consumption.

In this study participated a significant number of participants from Primorsko-goranska County in Croatia (81%) which makes the study results relevant and reliable. It found a significant proportion of participants that consumed fish and seafood products within recommendations and a higher proportion of those having a diet that moderately to highly adhered to the MD. This study provided the data on the average consumption of various fish and seafood regarding socio-demographic variables. Those data could serve for further analytical research regarding environmental pollutants in seafood and assessing the potential health risk for population groups. However, this study is limited to the fact that the consumption was assessed by a self-report measure that may be biased. Also, the data were collected from inhabitants of the coastal part of Croatia and they cannot be related to the habits and reasons of the inhabitants of other parts of Croatia. Another limitation is related to the data collection method applied. The study was processed by a self-fulfilling paper-type questionnaire, with an estimated participant's effort of 15–20 min, which represents a strong barrier to complete for people with low motivations. That limitation was controlled since this study included the data only from those questionnaires that were entirely responded to. However, future similar research should be directed toward a more objective measurement of fish and seafood consumption. Also, further detailed analysis of the nutritional profile of fish and seafood consumed could consider a comprehensive participants' nutrient profile originating from fish and seafood. This is important because there is a highlighted relationship between healthy and sustainable food, meals and diets (Hallström et al., 2018) which has not been investigated in Croatia so far.

Conclusion

The data obtained from this study showed an average consumption of fish and seafood according to different socio-demographic subgroups. More fish is consumed by women, the elderly, the more educated, non-smokers, and the more physically active participants. Given the recommended weekly intake of fish, a significantly larger proportion of participants reached these recommendations. That subgroup highlighted fish health benefits as the main reason for choosing to consume fish, but also the liking of its taste and the tradition of its consumption. Participants preferred the most wild-caught fish originating locally, regardless of the frequency of fish and seafood consumption. The obtained data are valuable because they cover the habits and reasons for fish and seafood consumption, which is valuable in terms of directing the sustainable diet promotion program, in which fish and seafood consumption have a significant part, not only for its proven health benefits but also for its beneficial environmental impact.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Teaching Institute of Public Health of Primorsko-Goranska County, Croatia. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SM, SP, LT, and MG designed the research. SM, GK, and ŽL analyzed the data. SM, GK, ŽL, MG, LT, and SP wrote the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abdelhamid, A. S., Brown, T. J., Brainard, J. S., Biswas, P., Thorpe, G. C., Moore, H. J., et al. (2018). Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 30, CD003177. doi: 10.1002/14651858.CD003177.pub4

Akbaraly, T. N., and Brunner, E. J. (2008). Socio-demographic influences on trends of fish consumption during later adult life in the Whitehall II study. *Br. J. Nutr*. 100, 1116–1127. doi: 10.1017/S0007114508971312

Almeida, C., Altintzoglou, T., Cabral, H., and Vaz, S. (2015). Does seafood knowledge relate to more sustainable consumption? *Br. Food J. Hyg. Rev*. 117, 894–914. doi: 10.1108/BFJ-04-2014-0156

Altintzoglou, T., Birch Hansen, K., Valsdottir, T., Oyvind Odland, J., Martinsdottir, E., Brunso, K., et al. (2010). Translating barriers into potential improvements: the case of new healthy seafood product development. *J. Consum. Mark*. 27/3, 224–235. doi: 10.1108/07363761011038293

Anderson, J. S., Nettleton, J. A., Herrington, D. M., Craig Johnson, W., Tsai, M. Y., Siscovic, D., et al. (2010). Relation of omega-3 fatty acid and dietary fish intake with brachial artery flow-mediated vasodilation in the Multi-Ethnic Study of Atherosclerosis. *Am. J. Clin. Nutr*. 92, 1204–1213. doi: 10.3945/ajcn.2010.29494

Appleton, K. M. (2016). Barriers to and facilitators of the consumption of animal-based protein-rich foods in older adults. *Nutrients*. 8, 187. doi: 10.3390/nu8040187

Belin, R. J., Greenland, P., Martin, L., Oberman, A., Tinker, L., Robinson, J., et al. (2011). Fish intake and the risk of incident heart failure: the Women's Health Initiative. *Circ. Heart Fail*. 4, 404–413. doi: 10.1161/CIRCHEARTFAILURE.110.960450

Belle, F., Wengenroth, L., Weiss, A., Sommer, G., Beck Popovic, M., Ansari, M., et al. (2017). Swiss paediatric oncology group (SPOG). Low adherence to dietary recommendations in adult childhood cancer survivors. *Clin. Nutr*. 36, 1266–1274. doi: 10.1016/j.clnu.2016.08.012

Béné, C., Barange, M., Subasinghe, R., Pinstrup-Andersen, P., Merino, G., Hemre, G. I., et al. (2015). Feeding 9 billion by 2050 – Putting fish back on the menu. *Food Security* 7, 261–274. doi: 10.1007/s12571-015-0427-z

Berry, E. M. (2019). Sustainable food systems and the mediterranean diet. *Nutrients*. 11, 2229. doi: 10.3390/nu11092229

Birch, D., Lawley, M., and Hamblin, D. (2012). Drivers and barriers to seafood consumption in Australia. *J. Consum. Mark*. 29, 64–73. doi: 10.1108/07363761211193055

Bishop, N., and Leblanc, C. (2017). Dietary intake of DHA and EPA in a group of pregnant women in the moncton area. *Can. J. Diet Pract. Res*. 78, 59–65. doi: 10.3148/cjdpr-2016-033

Bonaccio, M., Ruggiero, E., Di Castelnuovo, A., Costanzo, S., Persichillo, M., De Curtis, A., et al. (2017). Fish intake is associated with lower cardiovascular risk in a Mediterranean population: Prospective results from the Moli-sani study. *Nutr. Metab. Cardiovasc. Dis*. 27, 865–873. doi: 10.1016/j.numecd.2017.08.004

Bostic, S. M., Sobal, J., Bisogni, C. A., and Monclova, J. M. (2017). Types and characteristics of fish and seafood provisioning scripts used by rural midlife adults. *J. Nutr. Educ. Behav*. 49, 535–44.e1. doi: 10.1016/j.jneb.2017.03.005

Burlingame, B., and Dernini, S. (eds.) (2012). *Sustainable Diets: Directions and Solutions for Policy, Research and Action*. Rome: FAO.

Buscemi, S., Nicolucci, A., Lucisano, G., Galvano, F., Grosso, G., Belmonte, S., et al. (2014). Habitual fish intake and clinically silent carotid atherosclerosis. *Nutr. J*. 13, 2. doi: 10.1186/1475-2891-13-2

Cardoso, C., Bandarra, N., Lourenço, H., and Afonso, C. (2010). Methylmercury risks and EPA+DHA benefits associated with seafood consumption in Europe. *Risk Anal*. 30, 827–840. doi: 10.1111/j.1539-6924.2010.01409.x

Cardoso, C., Lourenço, H., Costa, S., Gonçalves, S., and Nunes, M. L. (2013). Survey into the seafood consumption preferences and patterns in the Portuguese population. Gender regional variability. *Appetite*. 64, 20–31. doi: 10.1016/j.appet.2012.12.022

Chakraborty, K., Joy, M., and Vijayagopal, P. (2016). Nutritional qualities of common edible cephalopods at the Arabian Sea. *Int. Food Res. J*. 23, 1926–1938.

Chen, J., Jayachandran, M., Bai, W., and Xu, B. (2022). A critical review on the health benefits of fish consumption and its bioactive constituents. *Food Chem*. 369, 130874. doi: 10.1016/j.foodchem.2021.130874

Christenson, J. K., O'Kane, G. M., and Farmery, A. K., and McManus, A. (2017). The barriers and drivers of seafood consumption in Australia: a narrative literature review. *Int. J. Cons. Stud*. 41, 299–311. doi: 10.1111/ijcs.12342

Crona, B., Wass'enius, E., Troell, M., Barclay, K., Mallory, T., Fabinyi, M.i., et al. (2020). China at a crossroads: an analysis of China's changing seafood production and consumption. *One Earth* 3, 32–44. doi: 10.1016/j.oneear.2020.06.013

de Roos, B., Sneddon, A. A., Sprague, M., Horgan, G. W., and Brouwer, I. A. (2017). The potential impact of compositional changes in farmed fish on its health-giving properties: is it time to reconsider current Dietary Recommendations? *Public Health Nutr*. 20, 2042–2049. doi: 10.1017/S1368980017000696

Dijkstra, S. C., Neter, J. E., Brouwer, I. A., Huisman, M., and Visser, M. (2014). Adherence to dietary guidelines for fruit, vegetables and fish among older Dutch adults; the role of education, income and job prestige. *J. Nutr. Health Aging*. 18, 115–121. doi: 10.1007/s12603-013-0402-3

Domingo, J. L. (2016). Nutrients and chemical pollutants in fish and shellfish. Balancing health benefits and risks of regular fish consumption. *Crit. Rev. Food Sci. Nutr*. 56, 979–988. doi: 10.1080/10408398.2012.742985

EFSA (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food, EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J*. 10, 2985. doi: 10.2903/j.efsa.2012.2985

EUMOFA (2019). *The EU Fish Market, 2019 Edition*. Publication Office of the European Union, 101. Available online at: https://www.eumofa.eu/documents/20178/314856/EN_The+EU+fish+market_2019.pdf/ (accessed February 14, 2022).

EUMOFA (2020). *The EU Fish Market, 2020*. Publications Office of the European Union, 101. Available online at: https://www.eumofa.eu/documents/20178/415635/EN_The+EU+fish+market_2020.pdf (accessed February 14, 2022).

FAO (2015). *International Centre for Advanced Mediterranean Agronomic Studies/Food and Agriculture Organization of the United Nations*. Available online at: https://www.oecd.org/gov/regulatory-policy/FAO_Full-Report.pdf (accessed January 29, 2022).

FAO (2020). *The State of World Fisheries and Aquaculture 2020*. Sustainability in action. Rome. 206p. Available online at: https://doi.org/10.4060/ca9229en (accessed March 13, 2022).

Food data (2019). *(frida.fooddata.dk), version 4, 2019*. National Food Institute, Technical University of Denmark. Available online at: https://frida.fooddata.dk/?lang=en (accessed March 25, 2022).

Giuli, C., Papa, R., Mocchegiani, E., and Marcellini, F. (2012). Dietary habits and aging in a sample of Italian older people. *J. Nutr. Health Aging*. 16, 875–879. doi: 10.1007/s12603-012-0080-6

Golden, C. D., Allison, E. H., Cheung, W. W., Dey, M. M., Halpern, B. S., McCauley, D. J., et al. (2016). Nutrition: fall in fish catch threatens human health. *Nature*. 16, 317–320. doi: 10.1038/534317a

Govzman, S., Looby, S., Wang, X., Butler, F., Gibney, E. R., Timon, C. M., et al. (2021). A systematic review of the determinants of seafood consumption. *Br. J. Nutr*. 126, 66–80. doi: 10.1017/S0007114520003773

Grieger, J. A., Miller, M., and Cobiac, L. (2012). Knowledge and barriers relating to fish consumption in older Australians. *Appetite*. 59, 456–463. doi: 10.1016/j.appet.2012.06.009

Hallström, E., Davis, J., Woodhouse, A., and Sonesson, U. (2018). Using dietary quality scores to assess sustainability of food products and human diets: a systematic review. *Ecol. Indicat*. 93, 219–230. doi: 10.1016/j.ecolind.2018.04.071

He, K., Liu, K., Daviglus, M. L., Jenny, N. S., Mayer-Davis, E., Jiang, R., et al. (2009). Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). *Am. J. Cardiol*. 103, 1238–1243. doi: 10.1016/j.amjcard.2009.01.016

Health Statistical Yearbook of Primorsko-Goranska County for 2020. (2022). *Distribution of Population by Sex and Age*. Croatia: Institute of Public Health of the Primorsko-goranska County. Available online at: https://www.zzjzpgz.hr/index.php?show=statistika (accessed January 29, 2022).

Hearty, A. P., McCarthy, S. N., Kearney, J. M., and Gibney, M. J. (2007). Relationship between attitudes towards healthy eating and dietary behavior, lifestyle and demographic factors in a representative sample of Irish adults. *Appetite*. 48, 1–11. doi: 10.1016/j.appet.2006.03.329

Heppe, D. H., Steegers, E. A., Timmermans, S., Breeijen, H.d., Tiemeier, H., Hofman, A., et al. (2011). Maternal fish consumption, fetal growth and the risks of neonatal complications: the Generation R Study. *Br. J. Nutr*. 105, 938–949. doi: 10.1017/S0007114510004460

Hostenkamp, G., and Sørensen, J. (2010). Are fish eaters healthier and do they consume less health-care resources? *Public Health Nutr*. 13, 453–460. doi: 10.1017/S1368980009991327

Jacobs, S., Sioen, I., Marques, A., and Verbeke, W. (2018). Consumer response to health and environmental sustainability information regarding seafood consumption. *Environ. Res*. 161, 492–504. doi: 10.1016/j.envres.2017.10.052

Jahns, L., Raatz, S. K., Johnson, L. K., Kranz, S., Silverstein, J. T., Picklo, M. J., et al. (2014). Intake of seafood in the US varies by age, income, and education level but not by race-ethnicity. *Nutrients*. 6, 6060–6075. doi: 10.3390/nu6126060

Kendel Jovanović, G., Pavičić ŽeŽelj, S., Klobučar Majanović, S., Mrakovcic-Sutic, I., and Šutić, I. (2020). Metabolic syndrome and its association with the Dietary Inflammatory Index (DII)® in a Croatian working population. *J. Hum. Nutr. Diet*. 33, 128–137. doi: 10.1111/jhn.12695

Koehn, J. Z., Allison, E. H., Golden, C. D., and Hilborn, R. (2022). The role of seafood in sustainable diets. *Environ. Res. Lett*. 17, 035003. doi: 10.1088/1748-9326/ac3954

Kolčić, I., Relja, A., Gelemanović, A., Miljković, A., Boban, K., Hayward, C., et al. (2016). Mediterranean diet in the southern Croatia - does it still exist? *Croat. Med. J*. 31, 415–424. doi: 10.3325/cmj.2016.57.415

Kossioni, A., and Bellou, O. (2011). Eating habits in older people in Greece: the role of age, dental status and chewing difficulties. *Arch. Gerontol. Geriatr*. 52, 197–201. doi: 10.1016/j.archger.2010.03.017

Langlois, K., and Ratnayake, W. M. N. (2015). Omega-3 index of Canadian adults. *Health Reports* 26, 3–11.

Larsson, S. C., Virtamo, J., and Wolk, A. (2011). Fish consumption and risk of stroke in Swedish women. *Am. J. Clin. Nutr*. 93, 487–493. doi: 10.3945/ajcn.110.002287

Lawley, M., Birch, D., and Hamblin, D. (2012). An exploratory study into the role and interplay of intrinsic and extrinsic cues in Australian consumers' evaluations of fish. *Austral. Mark. J*. 20, 260–267. doi: 10.1016/j.ausmj.2012.05.014

Levitan, E. B., Wolk, A., and Mittleman, M. A. (2010). Fatty fish, marine omega-3 fatty acids and incidence of heart failure. *Eur. J. Clin. Nutr*. 64, 587–594. doi: 10.1038/ejcn.2010.50

Lofstedt, A., de Roos, B., and Fernandes, P. G. (2021). Less than half of the European dietary recommendations for fish consumption are satisfied by national seafood supplies. *Eur. J. Nutr*. 60, 4219–4228. doi: 10.1007/s00394-021-02580-6

MARM (2010). *Consumo Alimentario en España*. Madrid: Ministerio de Medio Ambiente y Medio Rural y Marino e Instituto Cerdá. Available online at: http://www.marm.es (accessed March 29, 2022).

Martínez-González, M. A., García-Arellano, A., Toledo, E., Salas-Salvadó, J., Buil-Cosiales, P., Corella, D., et al. (2012). PREDIMED Study Investigators. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLoS ONE*. 7, e43134. doi: 10.1371/journal.pone.0043134

Marushka, L., Batal, M., Sadik, T., Schwartz, H., Ing, A., Fediuk, K., et al. (2018). Seafood consumption patterns, their nutritional benefits and associated sociodemographic and lifestyle factors among First Nations in British Columbia, Canada. *Public Health Nutr*. 21, 3223–3236. doi: 10.1017/S136898001800215X

Mazzocchi, A., Leone, L., Agostoni, C., and Pali-Schöll, I. (2019). The secrets of the Mediterranean diet. Does [Only] olive oil matter? *Nutrients*. 11, 2941. doi: 10.3390/nu11122941

McGuire, S. (2016). Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Washington, DC: US Departments of Agriculture and Health and Human Services, 2015. *Adv. Nutr*. 7, 202–4. doi: 10.3945/an.115.011684

McManus, A., and Merga, M. (2011). Omega-3 fatty acids. What consumers need to know. *Appetite* 57, 80–83. doi: 10.1016/j.appet.2011.03.015

Meier, M., Berchtold, A., Akré, C., Michaud, P. A., and Surís, J. C. (2010). Who eats healthily? A population-based study among young Swiss residents. *Public Health Nutr*. 13, 2068–2075. doi: 10.1017/S1368980010000947

Ministarstvo poljoprivrede, Uprava ribarstva, oŽujak (2021). *godine 2021. Dostupnost i vidljiva potrošnja proizvoda ribarstva i akvakulture u Republici Hrvatskoj u 2018. i 2019. godini*. Available online at: https://ribarstvo.mps.hr/UserDocsImages/Dostupnost%20i%20potro%C5%A1nja%20proizvoda%20ribarstva%20i%20akvakulture_FINALNI%20UR.pdf (accessed March 16, 2022).

Mouritsen, O. G., and Styrbæk, K. (2018). Cephalopod gastronomy—a promise for the future. *Front. Commun*. 3, 38. doi: 10.3389/fcomm.2018.00038

Mozaffarian, D., Stein, P. K., Prineas, R. J., and Siscovick, D. S. (2008). Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. *Circulation*. 117, 1130–1137. doi: 10.1161/CIRCULATIONAHA.107.732826

Murray, G., Wolff, K., and Patterson, M. (2017). Why eat fish? Factors influencing seafood consumer choices in British Columbia, Canada. *Ocean. Coast. Dev*. 144, 16–22. doi: 10.1016/j.ocecoaman.2017.04.007

Nesheim, M., Oria, M., and Yih, P. (2015). *A Framework for Assessing Effects of the Food System, 1st Edn*. Washington, DC: National Academies Press.

Nordgren, T. M., Lyden, E., Anderson-Berry, A., and Hanson, C. (2017). Omega-3 fatty acid intake of pregnant women and women of childbearing age in the United States: potential for deficiency? *Nutrients*. 26, 197. doi: 10.3390/nu9030197

Piepoli, M. F., Hoes, A. W., Agewall, S., Albus, C., Brotons, C., Catapano, A. L., et al. (2016). ESC Scientific Document Group. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention and Rehabilitation (EACPR). *Eur. Heart J*. 37, 2315–2381. doi: 10.1093/eurheartj/ehw106

Portugal-Nunes, C., Nunes, F. M., Fraga, I., Saraiva, C., and Gonçalves, C. (2021). Assessment of the methodology that is used to determine the nutritional sustainability of the mediterranean diet-a scoping review. *Front. Nutr*. 23, 772133. doi: 10.3389/fnut.2021.772133

Rahmawaty, S., Charlton, K., Lyons-Wall, P., and Meyer, B. J. (2013). Factors that influence consumption of fish and omega-3-enriched foods: a survey of Australian families with young children. *Nutr. Dietet.* 70, 286–293. doi: 10.1111/1747-0080.12022

Sacchettini, G., Castellini, G., Graffigna, G., Hung, Y., Lambri, M., Marques, A., et al. (2021). Assessing consumers' attitudes, expectations and intentions towards health and sustainability regarding seafood consumption in Italy. *Sci. Total Environ*. 789, 148049. doi: 10.1016/j.scitotenv.2021.148049

Sala-Vila, A., Harris, W. S., Cofán, M., Pérez-Heras, A. M., Pintó, X., Lamuela-Raventós, R. M., et al. (2011). Determinants of the omega-3 index in a Mediterranean population at increased risk for CHD. *Br. J. Nutr*. 106, 425–431. doi: 10.1017/S0007114511000171

Šarac, J., Havaš Auguštin, D., Lovrić, M., Stryeck, S., Šunić, I., Novokmet, N., et al. (2021). A generation shift in mediterranean diet adherence and its association with biological markers and health in Dalmatia, Croatia. *Nutrients*. 13, 4564. doi: 10.3390/nu13124564

Serra-Majem, L., Román-Viñas, B., Sanchez-Villegas, A., Guasch-Ferré, M., Corella, D., La Vecchia, C., et al. (2019). Benefits of the Mediterranean diet: epidemiological and molecular aspects. *Mol. Aspects Med*. 67, 1–55. doi: 10.1016/j.mam.2019.06.001

Sotos-Prieto, M., Santos-Beneit, G., Pocock, S., Redondo, J., Fuster, V., Peñalvo, J. L., et al. (2015). Parental and self-reported dietary and physical activity habits in pre-school children and their socio-economic determinants. *Public Health Nutr*. 18, 275–285. doi: 10.1017/S1368980014000330

Springmann, M., Spajic, L., Clark, M. A., Poore, J., Herforth, A., Webb, P., et al. (2020). The healthiness and sustainability of national and global food based dietary guidelines: modelling study. *BMJ*. 370, m2322. doi: 10.1136/bmj.m2322

Sveinsdottir, K., Martinsdottir, E., Green-Petersen, D., Hyldig, G., Schelvis, R., Delhunty, C. (2009). Sensory characteristics of different cod products related to consumer preferences and attitudes. *Food Qual. Prefer*. 20, 120–132. doi: 10.1016/j.foodqual.2008.09.002

Thong, N. T., and Solgaard, H. S. (2017). Consumer's food motives and seafood consumption. *Food Qual. Prefer*. 56, 181–188. doi: 10.1016/j.foodqual.2016.10.008

Tomić, M., Matulić, D., and Jelić, M. (2016). What determines fresh fish consumption in Croatia? *Appetite*. 106, 13–22. doi: 10.1016/j.appet.2015.12.019

Trondsen, T., Scholderer, J., Lund, E., and Eggen, A. E. (2003). Perceived barriers to consumption of fish among Norwegian women. *Appetite* 41, 301–314. doi: 10.1016/S0195-6663(03)00108-9

van Woudenbergh, G. J., van Ballegooijen, A. J., Kuijsten, A., Sijbrands, E. J., van Rooij, F. J., Geleijnse, J. M., et al. (2009). Eating fish and risk of type 2 diabetes: a population-based, prospective follow-up study. *Diabetes Care*. 32, 2021–2026. doi: 10.2337/dc09-1042

Verbeke, W., Sioen, I., Pieniak, Z., Van Camp, J., and De Henauw, S. (2005). Consumer perception versus scientific evidence about health benefits and safety risks from fish consumption. *Public Health Nutr*. 8, 422–429. doi: 10.1079/PHN2004697

Vilarnau, C., Stracker, D. M., Funtikov, A., da Silva, R., Estruch, R., Bach-Faig, A. (2019). Worldwide adherence to Mediterranean Diet between 1960 and 2011. *Eur. J. Clin. Nutr*. 72, 83–91. doi: 10.1038/s41430-018-0313-9

Vilavert, L., Borrell, F., Nadal, M., Jacobs, S., Minnens, F., Verbeke, W., et al. (2017)Health risk/benefit information for consumers of fish and shellfish: FishChoice, a new online tool. *Food Chem Toxicol*. 104, 79–84. doi: 10.1016/j.fct.2017.02.004

Virtanen, J. K., Mozaffarian, D., Cauley, J. A., Mukamal, K. J., Robbins, J., Siscovick, D. S., et al. (2010). Fish consumption, bone mineral density, and risk of hip fracture among older adults: the cardiovascular health study. *J. Bone Miner. Res*. 25, 1972–1979. doi: 10.1002/jbmr.87

Wallin, A., Di Giuseppe, D., Orsini, N., Åkesson, A., Forouhi, N. G., Wolk, A., et al. (2017). Fish consumption and frying of fish in relation to type 2 diabetes incidence: a prospective cohort study of Swedish men. *Eur. J. Nutr*. 56, 843–852. doi: 10.1007/s00394-015-1132-6

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet*. 393, 447–492. doi: 10.1016/S0140-6736(18)31788-4

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Consumer acceptance of new food trends resulting from the fourth industrial revolution technologies: A narrative review of literature and future perspectives

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The growing consumer awareness of climate change and the resulting food sustainability issues have led to an increasing adoption of several emerging food trends. Some of these trends have been strengthened by the emergence of the fourth industrial revolution (or Industry 4.0), and its innovations and technologies that have fundamentally reshaped and transformed current strategies and prospects for food production and consumption patterns. In this review a general overview of the industrial revolutions through a food perspective will be provided. Then, the current knowledge base regarding consumer acceptance of eight traditional animal-proteins alternatives (e.g., plant-based foods and insects) and more recent trends (e.g., cell-cultured meat and 3D-printed foods) will be updated. A special focus will be given to the impact of digital technologies and other food Industry 4.0 innovations on the shift toward greener, healthier, and more sustainable diets. Emerging food trends have promising potential to promote nutritious and sustainable alternatives to animal-based products. This literature narrative review showed that plant-based foods are the largest portion of alternative proteins but intensive research is being done with other sources (notably the insects and cell-cultured animal products). Recent technological advances are likely to have significant roles in enhancing sensory and nutritional properties, improving consumer perception of these emerging foods. Thus, consumer acceptance and consumption of new foods are predicted to continue growing, although more effort should be made to make these food products more convenient, nutritious, and affordable, and to market them to consumers positively emphasizing their safety and benefits.

KEYWORDS

alternative proteins, edible insects, cultured meat, consumer perception, plant-based food, 3D food printing, personalized nutrition, industry 4.0

Introduction

The global challenges for economic, social, and environmental sustainable development are currently more acute than ever before and call for immediate actions to develop a healthier and more sustainable future of food (1–3). Food production systems, mainly the production of animal-sourced food through livestock farming, have been a significant contributor to climate change and unsustainable development. Therefore, a search is underway worldwide to find alternative technologies and production methods that provide food with a lower environmental footprint while nutritional and sensory characteristics are similar or even better than that of animal products (4–8).

Plant-based sources have been investigated and established for use as food and feed throughout human development, but consumer interest in plant-based proteins has recently increased, which is reflected in a growing number of vegans, vegetarians, or flexitarians. A variety of plant-based meat, fish, milk, and egg analogs are being introduced to the market as a promising, sustainable approach to reduce the consumption of meat and other animal-based proteins (9, 10). While wild-harvested insects have been a traditional food source in many countries for centuries (11), insects' cultivation is relatively new, with some small-scale insect farming projects being launched in some countries (12). Apart from these traditional sources (i.e., plant-based foods and insects), other more innovative solutions, especially cell-cultured and 3D printed-foods, are being evaluated. Cell-cultured food production (e.g., meat, seafood, and poultry) is being studied owing to its potential to achieve environmental sustainability, due to low land and water requirements and reduced greenhouse gas emissions as well as improved animal welfare (13–15). 3D printing is a new technique that has become part of many scientific fields and industrial areas, including the food industry, allowing the production of on-demand, complex, and customized foods. In addition, the technique may be used for personalized diet (or personalized foods) to print products that specifically meet an individual's health-nutritional needs (16, 17). Another emerging application of 3D printing is cultured meat (18).

Innovative technologies have the potential to improve food production and enhance the quality of new food products to improve consumer acceptance. Gene editing is one of the emerging technologies that have opened up many possibilities for generating crops and animals with improved properties and desired traits (19–21). Additionally, highly productive food production systems (e.g., hydroponics, aquaponics, and aeroponics) have received attention as alternative farming systems, taking advantages of innovations and advancements in science and technology (22–24). Increased concerns about environmental sustainability are driving the growing interest in better uses of food wastes, by-products, and ugly produce. Food wastes is one of the major challenges for the global food system as approximately one-third of food produced in the world for human consumption is either lost or wasted each year. Valorization of food by-products and ugly produce (e.g., food products with an abnormal appearance) using smart solutions and technologies can constitute a promising strategy to tackle this challenge (25–27).

The evolution of consumers' demands for the aforementioned eight food trends, namely, plant-based, insect-based, cell-based, 3D-printed, personalized, and gene-edited foods, as well as foods resulting from by-products and ugly produce and new production systems (Figure 1) has resulted in a complexity that requires advanced technologies and innovative solutions. There is a growing literature on these selected food trends as can be seen in Figure 2. These trends have been further fueled by recent technological innovations accompanied by the advent of the fourth industrial revolution (or Industry 4.0) technologies. Due to its complexity, it is difficult to provide a single, concise definition of Industry 4.0 that will be universally accepted. However, Industry 4.0 can be seen as a combination of smart and advanced technologies in the digital, physical, and biological fields that enables more advanced intelligence to be brought to manufacturing and the transition from mass to customized production (28, 29). The main Industry 4.0 enablers in the food industry include

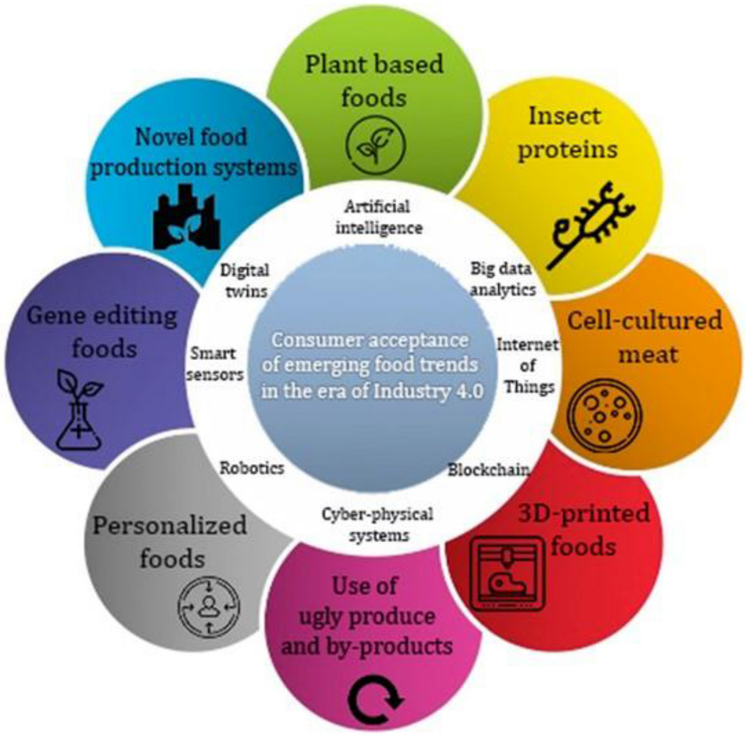

FIGURE 1
The food trends reviewed in this manuscript and the main enablers of the fourth industrial revolution.

artificial intelligence (AI), big data, the Internet of Things (IoT), blockchain, smart sensors, robotics, digital twins, and cyber-physical systems (30, 31).

Plant-based foods have been thoroughly reviewed in recent publications (10, 32–35). Detailed review papers reporting on insects protein (11, 36–38), cell-cultured (13–15), and 3D printed (39–41) food products have also been published. Other publications reported on personalized diet (16, 17, 42, 43), gene editing technologies (19, 20, 44), valorization of food by-products and ugly produce (45–48), and new food production systems (22, 24, 49). However, the applicability of Industry 4.0 concepts with each of these food trends has not been reviewed. Therefore, the main objective of this narrative review is to highlight the important scientific and technological advances that are being used to improve sensory, nutritional, and technological qualities of emerging food trends (shown in Figure 1), and enhancing their acceptance by consumers.

Overview of the industrial revolutions through a food perspective

The ever-increasing population is putting pressure on natural resources and depleting them rapidly. Technological, environmental, social, and political changes across the globe are creating many new opportunities and challenges for humans. The global population increase is significantly affecting food and water sources (50). The overall industrial revolutions have had a great impact on the many different components of the food industry. The food industry has been continuously updating its processes and products to meet each new revolution (51). Industry 4.0 comprises a diversity of new enabling technologies, as previously mentioned that includes smart sensors, big data, AI, IoT, blockchain, cloud computing, automation, among others. These technologies have important roles in creating modern production processes. The food industry is adopting a customer-orientation as part of a dynamic supply chain. An adaptation of innovative technologies in the different food sectors is important for the sustainability of the production process. New technologies are often also more efficient economically (52, 53).

The United Nations is striving to make the environment and food production more sustainable for upcoming generations. The industrial revolutions are important factors for sustainable food production and the environment (54–56). Industry 4.0 has a direct impact on food manufacturing and the food supply chain. Industry 4.0 is integrating human actors and intelligent machines with product and process lines. Consumer demand for healthy food will best be provided by adopting Industry

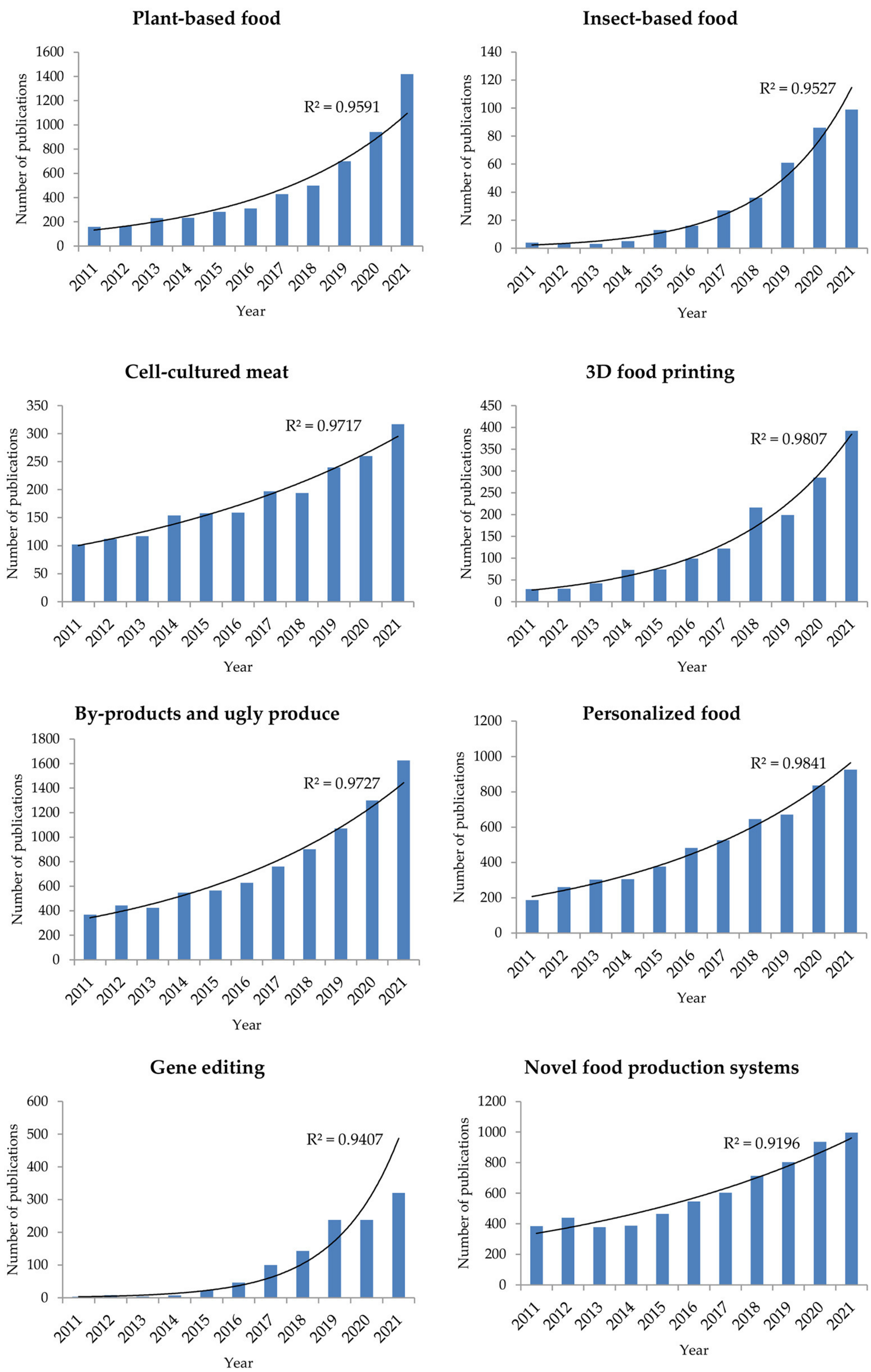

FIGURE 2
Number of publications per year (until March 2022) dealing with emerging food trends (data obtained from Scopus).

4.0 changes. For example, thermal food processing is being replaced with non-thermal technologies to minimize nutrient losses (56). The use of non-thermal technologies (e.g., high-pressure processing (HPP), cold plasma (CPL), and pulsed UV-light) are improving processing to produce safe and more nutritious food products (57).

Automation is having an increasingly important role in manufacturing to achieve maximum productivity. The use of AI with automated processes is increasing in the food industry (54). AI helps monitor the supply chain and overall production process. IoT includes many technologies that will also affect existing production processes. IoT could also be implemented in the food supply chain to make food safer (58). IoT connects different devices to ensure effective communications between people and things (59). The use of sensor technology and cloud computing devices are important in increasing the efficiency of the food supply chain (60, 61). The application of Industry 4.0 technologies (e.g., IoT, blockchain, and smart sensors) is also important in reducing food wastage (62). Generally, the digital revolution currently occurring in manufacturing and the food industry, accompanied by greater automation and advanced monitoring methods and processing technologies is likely to have significant roles in enhancing sensory quality and nutritional properties of foods, leading to improved consumer perception and acceptance of these foods.

Consumer acceptance of emerging food trends

Plant-based foods

Current food production practices have been linked to a high prevalence of various chronic diseases as well as significant environmental damage (63, 64). Over the past century, the modern food and agricultural sectors have contributed to a considerable reduction in world malnutrition and hunger by producing a bountiful supply of inexpensive, safe, and tasty foods. To feed a rising and wealthier global population, more food of higher quality is required. Large-scale production of animal products such as milk, fish, meat, eggs, and their derivatives have been identified as a major contributor to the modern food supply's negative impact on global environmental sustainability (63). Raising cattle for food causes significantly more pollution, water and land use, greenhouse gas emissions, and biodiversity loss than growing plants (and in some cases other animals) for human use (65).

Plant-based (PB) diets are becoming increasingly popular as a strategy to lessen the diet's environmental footprint while simultaneously improving human health and animal welfare. In comparison to omnivores, vegetarians and vegans make up a small percentage of the population, but their numbers have risen in recent years. A side from meat alternatives, non-animal food products are becoming more popular, which creates a business opportunity for the food industry (66). Concerns over the consumption of animal-based food products and their harmful effects on the environment and health have led to an increase in the PB protein business, particularly for innovative items that can replace traditional dairy, egg, and meat products. More people are declaring themselves "flexitarians (vegetarians who occasionally eat animal products)," or opting to consume less dairy, eggs, and meat in favor of more PB meals to help the environment, improve health, or both. According to consumer market research, up to 5 million Americans will have given up meat totally between 2019 and 2020, becoming vegetarians or vegans (67) although data confirming this is not yet available.

Functional PB foods are produced from unprocessed or natural, as well as biotechnologically modified plants. They are considered to have a significant impact on health and wellbeing by reducing disease risks. Many of these functional foods have been related to lower incidences of a variety of health conditions, including diabetes, cardiovascular disease, gout, and cancer. As a result, there is rising interest in functional PB food research and development (68, 69). Individual PB foods, such as nuts, vegetables, fruits, legumes, whole grains, and coffee, have been shown to be beneficial to the cardiovascular system (70). Significant evidence, on the other hand, links particular animal foods, such as processed and red meat, to an elevated risk of cardiovascular diseases (71, 72), although these results remain controversial. Consumers are increasingly turning to PB milk replacements for health reasons such as lactose intolerance, cow's milk protein allergies, or as a lifestyle choice. PB milk substitutes are generally water-soluble extracts of oilseeds, legumes, pseudo-cereals, or cereals that resemble bovine milk in appearance. As a substitute for cow's milk, they are manufactured by reducing the raw material's size, extracting it in water, and then homogenizing it. Cow's milk replacers can be used as a straight replacement for cow's milk or in some animal milk-based recipes (73) although their nutritional profiles may be quite different and this remains a concern.

Furthermore, there is growing concern that animal waste lagoons and industrial meat production runoff would pollute natural resources such as rivers, streams, and drinking water although manure also represents a potential natural fertilizer. There is also concern that excessive livestock farming may result in the loss of critical carbon sinks such as forests and other regions, as well as increased greenhouse gas emissions, which will exacerbate current environmental and climate-related issues. For human health and natural resources reasons a sustainable food system that shifts the world population toward less animal-based foods and more PB foods is potentially beneficial. Dietary patterns rich in minimally processed whole grains, vegetables, fruits, nuts, and legumes have been recommend for increased sustainability and human health. Meanwhile, a variety of other PB food products have been developed to replace traditional animal-based foods such as meat alternatives, e.g., sausages, burgers, and other meat-like products made primarily from highly processed PB components. Even though these products provide more PB alternatives, they may or may not be intended to imitate the sensory experience of eating meat (65, 71, 72).

The number of people consuming PB diets is rapidly expanding, according to many vegan organizations and consulting firms, although some recent reports suggest that the rate of growth of this market segment may be slowing down as repeat purchases decrease. It is claimed that vegans in the United States increased by 500% from almost 4 million in 2014 to 19.6 million in 2017 (66). According to a national survey done in the United States in 2018, Americans had been reducing their meat consumption in the previous 3 years (74). In the United Kingdom, flexitarians account for 21% of the population, whereas vegetarians and vegans account for one in every eight people. In Germany, vegetarians went from 1% (2005) to 7% (2018); the meat-free population expanded by 94.4% from 2011 to 2016 in Italy, and flexitarians increased by 25% in 2 years in Spain (66). Furthermore, according to global research done in 2019, 40% of consumers are attempting to reduce animal protein consumption, with 10% having completely stopped eating red meat (75). PB meat substitutes are predicted to expand in value from USD 1.6 billion in 2019 to USD 3.5 billion by 2026. The top-selling meat replacement foods in 2019 were burgers (USD 283 million), hot dogs and sausages (USD 159 million), and patties (USD 120 million). Other figures show that sales of meat in the United States fell by 5% between 2015 and 2019 (66, 75).

Due to an increase in information about chronic diseases and the numerous health claims presently associated with various foods, consumer interest in wellness and better health is expanding. Nowadays, many consumers drink PB milk substitutes because they want to rather than because they have an allergy (76). PB milk substitutes are often regarded as healthy, owing to their established health claims, such as those relating to vitamins, fiber, or no cholesterol. The market is being driven by both these positive attributes as well as people's negative perceptions about cow's milk. The possibility of cow's milk contributing to a variety of human ailments, as well as its high-fat content, are among the concerns (76). The market for PB milk replacements has also grown significantly, more than tripling its global sales from 2009 to 2015 and reaching 21 billion USD (73). According to the Plant Based Foods Association, sales of PB yogurts have increased by 55%, PB cheeses by 43%, and PB creamers by 131% in the United States (66).

The Industry 4.0 food processing technologies improve functional, nutritional, and sensory properties of new PB foods. Non-thermal technologies such as PEF, HPP, high-pressure homogenization, and ultrasound modify the permeability of the cell membrane in numerous fruits and vegetables. This has been connected to microstructural changes in the whole matrices and reduced particle size in liquid matrices. In general, this increases the bioavailability of phenolic and carotenoids compounds by promoting their release (77). Furthermore, this type of processing might be effective in addressing the obstacles that come with processing PB drinks on a larger scale (69). An innovative drying processing technique–intermittent drying, is a method of changing the drying conditions by varying the humidity, temperature, pressure, velocity, or even the heat input mode. Longer drying durations and case hardening decrease energy efficiency, and lead to poor quality attributes that have been successfully addressed with this drying procedure in different PB foods (78).

The 3D-printing of PB foods has the potential to produce better quality PB foods. The purpose of 3D printing is to turn a computer-aided design model into a three-dimensional object. 4D printing is a relatively new technology that complements 3D printing by allowing the printed material to alter over time. Food 3D printing has the unique ability to create geometrically complex structures that can be mass produced while also saving money and the environment. It allows for the customization of foods based on nutritional needs, calorie consumption, texture, a precise shape, flavor, or color. For example, extrusion, selective sintering, binder jetting, and inkjets are the four types of 3D food printing technologies currently being studied for PB foods (79).

Insect-based foods

In response to the increase in the world's population, the existing production of food will have to treble to fulfill the rapidly rising demand for food. Insects are being researched as a new source of animal feed and human food to help meet global food security challenges. Human consumption of insects has several reported advantages including comparable protein levels (80), relatively high levels of unsaturated fat and different nutrients, and a lesser environmental effect due to decreased greenhouse gas emissions (80, 81). Insects are regarded as more sustainable since they utilize fewer natural resources such as water, feed, and land, and they generate far fewer greenhouse gases and ammonia than bovine and non-bovine animals. They have a high feed conversion ratio because they are cold-blooded, implying that they are particularly efficient at bio-transforming organic resources into insect biomass (82, 83).

As a result, insect production for human food is increasing in several countries (84). Around 2,000 edible insect species have been identified worldwide. They have been collected from the wild including from Africa, East Asia, and South America, and are used in traditional diets (37). Beetles (31%), caterpillars (18%), ants, wasps, and bees (14%), cricket, locusts, and grasshoppers (Orthoptera) (13%), planthoppers, cicadas, scale insects, true bugs (Hemiptera), and leafhoppers (10%), termites (Isoptera) (3%), dragonflies (Odonata) (3%); flies (Diptera) (2%); and other orders (5%) are the most commonly consumed species globally (85). For example cricket powder was added to pasta to increase its content of protein and minerals and improved the culinary properties and texture (86).

Insect-based food production has been influenced by recent advances and innovations offered by Industry 4.0 technologies. One of them is exploiting the possibilities for engineered insect tissue in cellular culture. Cellular agriculture is a rapidly emerging field that allows for the preparation of such a food system without necessitating changes in customer behavior. The use of insect cell culture in cellular agriculture offers the promise to overcome technical limitations and produce low-input, high-volume, and nutritious food. Insect cells are good candidates for incorporation into cultured meat and other innovative food products due to the robustness of established techniques for culturing insect cells and their ease of immortalization, serum-free growth, have a high-density proliferation, transfection, and a good suspension culture adaptation compared to mammalian cells (87).

Despite their apparent feasibility as a long-term alternative to conventional protein sources, there are still several barriers to their widespread utilization as human food in the West (81). In many Western countries, consumer acceptance remains a hurdle, and insects are usually viewed as unpleasant, even though their flavor has been shown to be mild and tolerable. Consumer disgust can be explained in numerous ways, including social, cultural and religious reasons (84). Therefore, product development to create new insect-based foods, as well as acceptance-boosting strategies, are required (88).

Consumer acceptance of insect-based food has been studied (89–91). For example, food neophobia, or a fear of trying new foods, emerges as an evolutionary response to prevent potential hazards from being tried. Many aspects of human eating behavior, including dietary preferences and food choices, are influenced by this attitude. Consumers in countries where there has been no recent insect intake history have a particularly neophobic attitude toward edible insects, which influences their apprehension to consume unusual and perhaps repulsive foods like insects (89). For example, the Chinese had more favorable attitudes and reported a higher willingness to eat insects compared to the Germans (90). Sensory aversion was discovered to be one of the commonly recognized risks of insect intake, which affects both Indians and Americans (89). Consumer acceptance of insect-based foods remains a hurdle in many cultures, where religious prohibitions rule out many insects. The role of context (social companions and location) in the acceptance of insect-based foods was studied (91). The results showed that eating with friends and eating in pubs enhanced the acceptance of insect-based foods. In another study (92), names and visual presentations were found to be important factor that affect consumer acceptance of insect-based foods.

Many strategies have been suggested to reduce food neophobia and increase acceptance of insect-based foods, among which processing seems to be the most promising (38, 83). Appropriate food processing and preparation techniques must be designed and implemented to obtain the benefits of insects. At all levels, including large-scale industrial, restaurants, cottage industries, and professional cuisine, as well as households, the processing is an important aspect of any meal or food item for the assimilation of insects into more standard cuisines. Processing preserves or improves the nutritional, organoleptic (texture, aroma, taste, etc.), and functionality of raw materials converted into food ingredients, while also destroying or removing potential safety hazards (93). Insect biomass processing is becoming increasingly necessary to meet the safe edible biomass standards while also developing effective techniques to reduce chemical and biological risks. Current insect biomass processing technologies rely on thermal (blanching, drying, boiling, freezing, chilling, and freeze-drying), mechanical (crushing, grinding, and milling), and fractionation processes (extraction, separation, purification, and centrifugation). These are well-developed and well-established in the food and feed industries (94). For example, the use of cold atmospheric pressure plasma processing in the postharvest chain for edible insects could aid in the creation of safe and high-quality insect-based products for the food and feed industries (95). Various food processing technologies such as oven, smoke, conventional air, freeze, microwave-assisted, and fluidized-bed drying methods, and ultrasound-assisted aqueous extraction, sonication, supercritical CO_2 extraction, and dry fractionation can be used to improve the overall quality of insect-based products as well as helping in extracting nutritionally rich compounds from the insects for its application in developing new food products (96).

Cell-cultured meat

To satisfy the growing demand for protein for an ever-increasing population, cultured meat is being considered a good substitute for meat. Cellular agriculture is an emerging field for the production of different products. Cultured meat, also known as clean meat or laboratory-grown meat, is a part of cellular agriculture and does not involve any livestock for the production of meat once the initial cells are obtained (13, 97, 98) although at some point new initial cells are needed.

Cell-cultured meat is produced using tissue-engineering techniques. Different aspects of cultured meat give it an edge over traditional meat such as a lower use of environmental resources, higher nutritional value, lower risk of food-borne diseases, as well as avoiding issues associated with the slaughtering of animals (13, 99) In the cell-cultured meat process, a biopsy is taken from any living animal from which the stem cells are obtained. The stem cells can proliferate into different types of cells. These cells are cultured in a nutrient medium containing all the required growth factors, nutrients, and hormones. The cells, if directed to muscle growth, continue to grow and form myotubes with a length of about 0.3 mm.

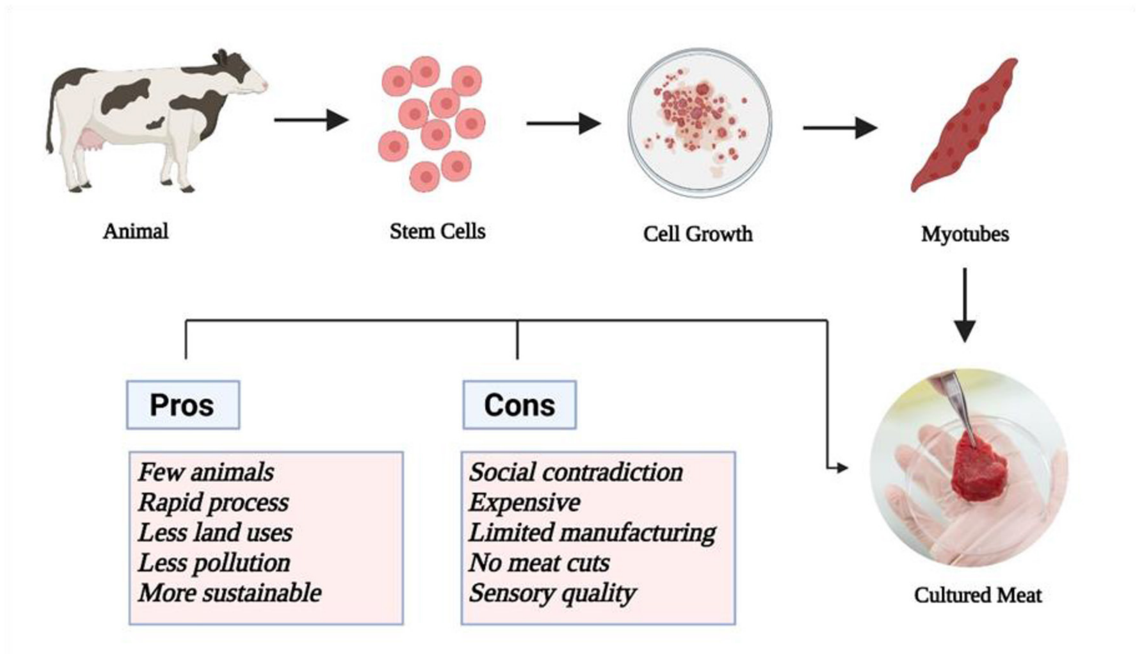

FIGURE 3
Process for the production of cellular meat with its pros and cons.

These myotubes are then placed in a ring that grows into a small piece of muscle tissue. A schematic diagram for their production is shown in Figure 3. These muscle tissues can further multiply to form more than a trillion strands. These muscle cells continue to grow in size and need to be attached to a scaffold that provides support and orientation (14, 100, 101).

The production process for cultured meat has various pros and cons. Cultured meat requires only a few animals to produce a large amount of meat through cell proliferation. The production process for laboratory-grown meat is rapid compared to natural processes. In addition, cell-cultured meat offers promising sustainability benefits. However, the cultured meat uses the blood of dead calves which is a controversial societal issue and negates the claims of being animal free and violates the religious traditions that do not permit the ingestion of blood or blood derivatives. The second major issue is that the use of this serum is expensive and increases the cost of production of laboratory grown meat. Currently only a limited range of meat cuts are available. Furthermore, the sensory quality of the meat is naturally affected by the type of animal including breed, growing conditions, feed, and many other factors, and to date has not fully imitated the flavor of the product it means to imitate. Additionally, laboratory-grown meat does not offer such diversification in terms of sensory quality. Therefore, there is a need to resolve the technical issues in the production of cultured meat. Additionally, there is a need to understand the safety aspects, optimization of cell culture methodology, and increase consumer acceptability. The acceptability of cultured meat by some religious authorities is still in question (14, 102, 103). A recent study showed that 35% of meat-eaters and 55% of vegetarians felt disgusted by cultured meat, as it is perceived as not being natural (104).

Despite the current limitations, the cell-cultured meat industry has recently been growing, especially in the past two years, with many companies being founded in North America, Asia, and Europe (105). Emerging innovations and Industry 4.0 technological advances (e.g., advances in biotechnology and 3D printing) are driving this trend, making it possible to accelerate the industrialization and commercialization process for cell-based products (4, 105). Among Industry 4.0 components, the role of 3D printing has been particularly highlighted, leading to many applications in different manufacturing fields, including cultured meat production (18, 106).

3D-printed foods

3D printing is being positively applied in different sectors of food production. The basic objective of 3D food production is to provide a highly structured food to the consumer. The main 3D food applications are based on the use of alternative ingredients, including different isolates from microorganisms, insects, food waste, and algae (107, 108). 3D printing can even be used to give a second life for plastic wastes (109), making it a promising

TABLE 1 Different types of 3D printed foods and consumer perception.

Product	Consumer response	Reference
Chocolates	The texture is too soft	(116)
	Higher levels of cocoa solids	
	Deliciousness	
	A very clever way of presenting tasty food	
Capsaicin candy	Nutritionally rich	(117, 118)
	Different shapes	
	Different flavors	
	Suitable for everyone	
	Helps in the prevention of numerous diseases	
Various types of meat products	Delicious	(18, 119)
	Different sizes	
	Rich protein source	
Pizzas	Highly delicious	(120)
	Healthiness	
Beef meat, hybrid meat analogs	3D printed meat	(18, 121, 122)
	Sustainable	
	Environmentally friendly	
	More durable	
	High cost	
	Potentially unappealing	

approach for achieving sustainability and circular economy goals for food packaging. The major research on 3D food printing is being done in the USA, China, and Australia (108, 110, 111). The first use of 3D was for the creation of engineering prototypes, while the first 3D food was introduced commercially in 2015 (108, 112, 113). Presently, many technologies for 3D food printing are being used (39, 114, 115).

Different types of 3D-printed foods are available as shown in Table 1. The major 3D printed food-producing countries are China, the United Kingdom, Canada, Spain, the United States, and Poland (108). Three important categories of ingredients for food printing include native non-printable, printable, and alternative ingredients. The native printable materials (e.g., chocolate, icing, and butter) can be extruded from a syringe. In the case of non-printable traditional food materials (e.g., fruits vegetables, meat, and rice) different viscosity enhancers (e.g., starches, gums, and gelatin) are added after grinding for a smooth extrusion process. Proteins and fibers isolated from insects, agricultural waste, and algae are considered alternative ingredients and have different functional properties (108, 110, 113).

3D food printing technologies are producing demand-based foods that can address food-related diseases (e.g., diabetes and obesity) and personal nutritional habits (e.g., vegetarian and vegan). 3D food printing technologies can also have a role in the production of customized food products, eliminating undesirable substances, and making foods that are pleasant for the consumer. Other advantages include food waste reduction, innovation, and process digitalization (17, 107, 108, 110, 123).

3D printing can be considered one of the most important enablers of Industry 4.0. Recent advances and technological developments have accelerated innovation and strengthened the use of 3D printing for different applications. Improvements in simulation, modeling, software, and materials have led to the extension of 3D printing to 4D, 5D, and 6D printing (41). 4D food printing refers to the response of 3D-printed foods integrated with smart materials to external or internal environmental/human stimuli (e.g., temperature and pH), resulting in physical or chemical changes (e.g., color, flavor, or nutritional changes) in the products over time (17, 40, 41). An example of the application of 4D printing in food was recently given by Ghazal and others (124) who used red cabbage juice and vanillin powder for their 4D product to change color and flavor in response to an external or internal pH stimulus. Recently, more advanced and innovative printing technologies, including 5D and 6D printing have emerged, presenting new possibilities in food manufacturing (41). Compared to 3D printing that is based on three axes (X, Y, and Z) of movement, 5D printing allows products to be printed from five axes by adding two additional rotational axes (i.e., the rotation of extruder head and the rotation of print bed), enabling printing of complex shapes having curved surfaces. 6D printing combines 4D and 5D printing techniques, making it possible to print complex structures using smart materials (41).

However, there are many issues associated with the production of printed foods. Among different issues, the unusual appearance of 3D food is a significant concern. The acceptability of the 3D-printed foods is another important challenge that should be addressed (108). Several survey-based studies were not always encouraging (120). In addition, the safety aspects of 3D-printed food must be addressed. Production of 3D foods includes cooling and heating which make the food more susceptible to microbial growth. The sanitization process for the printer is important to reduce the microbial load in the final product (110, 114).

The market for 3D food printers is expanding for the production of various types of foods. This technology has various advantages in terms of health, economic, and environment aspects, with a potential to revolutionize food manufacturing. 3D printing technology could be a way to alleviate hunger through a more efficient use of the available foods and the use of alternative food sources. Further improvement in functional and nutritional properties of printed foods is expected with the advent of Industry 4.0 innovations, enhancing consumer acceptance. However, before large scale commercialization, the consumer confidence and safety aspects of 3D-printed food must be addressed.

Use of by-products, ugly produce, and other sources (e.g., seaweeds and jellyfish) as food

Extensive research has been done to investigate new approaches to valorize food wastes and by-products and to explore new sources of food. Due to the growth of population and economic advances, larger amounts of agricultural and food wastes are produced at different stages of food production and consumption, causing different environmental problems (125, 126). Food wastes resulting from different food groups along the food supply chain were assessed and results showed that cereals, fruit, and vegetables were the food groups responsible for the highest amount of food wastes that occurred especially at the consumption stage (127).

Food waste valorization has garnered global attention as an effective approach in line with circular economy principles. Different strategies have been developed to reduce the waste resulting from the food industry and transform these wastes into resources (125, 128, 129). Food wastes and by-products can be rich in bioactive compounds and present important economic and environmental benefits. Different functional compounds may be extracted from food wastes and redirected to the food industry as ingredients or value-added compounds, to enrich products (47, 130). Proteins and amino acids, carotenoids and tocopherols, fatty acids, starches, oligosaccharides, soluble fibers, flavonoids, aromatic compounds, and different vitamins are examples of functional compounds extracted from different by-products and "ugly" produce and used to enrich different foods (131–134).

However, most of the current waste valorization strategies are developed only at laboratory scale (127). Additionally, consumer acceptance remains one of the main barriers that prevent commercialization of products and compounds extracted from food wastes and ugly produce. Research shows that abnormal appearance and nearing expiration date of food products can reduce consumer willingness to accept these products (48). In a recent study, the main drivers of intention to purchase products with a by-product, namely grape pomace powder, were evaluated (135). The results indicated that informing consumers positively of the presence of this by-product in food formulation enhanced the consumer acceptance of the product.

Jellyfish and seaweed have been highlighted in many studies as potential future foods (136–145). Jellyfish are marine invertebrates that are capable of growing in various environments (such as cold and warm waters, along coastlines, and in deeper waters) to form large blooms (146, 147). Interestingly, many reports indicated that the availability of jellyfish seems to increase with climate change, such as global warming (137, 147). These sustainable marine bioresources are valued for their reported health benefits showing high potential for use in food, feed, pharmaceutical, and other biotechnological applications, promoting their cultivation (146). Seaweeds are plant-like organisms that belong to brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta) algae (147, 148). These renewable sources of food have gained increased research and consumer interest in recent years due to their nutritional properties (e.g., high content of proteins, vitamins, minerals, and bioactive compounds) and their sustainability characteristics (e.g., fast growing with no fertilizer or pesticides), making them significant contributors to global food security (141, 142, 147).

Valorization of food wastes and by-products and exploitation of novel food sources take advantage of recent technological advances and the rapid spread of the concept of Industry 4.0. IoT, digital technologies, such as AI and digital twins, and other Industry 4.0 components are being applied to reduce or valorize food wastes and by-products, providing important environmental and economic benefits (62, 149–152). For example, emerging innovations in the field of algae biotechnology enable the development of low-cost production with exciting opportunities of automation through the application of IoT and other technological advances (145).

Developments in nanotechnology have provided many promising applications in the food industry, such as the use of food wastes and by-products in different sustainable food packaging strategies (153). Nanotechnology was used to reduce wine waste in obtaining new food ingredients and sustainable packaging with improved stability and bioavailability of the phenolic compounds (154). Grape pomace and broken wheat were used as printing material to produce functional cookies with enhanced nutritional value and antioxidant properties (155). The results showed that this sustainable approach led to food products with customized shapes and a higher content of proteins and dietary fiber.

Most of the extraction methods that are available industrially possessed several bottlenecks, such as using strong acidic solutions and high temperatures, including boiling water, leading to negative impacts on the sensory and nutritional quality of the extracted compounds and decreased consumer acceptance. Moreover, these extraction methods depend on different factors such as solvent properties, reaction temperature, pH, time of reaction, and the ratio between solvent and solid material (156–158). One the other hand, emerging green technologies, such as supercritical fluids, cold plasma, pulsed electric field, ultrasound, and high pressure processing have been studied and suggested as alternatives to conventional extraction methods. These techniques have a high potential to improve or maintain sensory and nutritional properties of foods, thus increasing their positive perception by consumers (25, 125, 158).

Personalized diet

A person's state of health can be improved through an individualized or personalized dietary approach. Healthy dietary choices may help to substantially reduce the occurrence of obesity and non-communicable diet-related diseases such as cancer, type-2 diabetes mellitus, metabolic syndrome, and cardiovascular diseases (159). Existing policy emphasizes prevention through personalized health promoting interventions, which have been shown to be successful in changing healthy behavior of consumers (160, 161). Digital technologies sustain individualized health promoting interventions by providing a personalized approach to health supporting activities that are easily accessible and cost effective (161). With the current breakthroughs in the decoding of the human genome and various applications of genomics, epigenomics, proteomics and metabolomics in medicine and nutrition, the modern personalized diet goes far beyond previous customized nutritional advice based on diet, age, sex, body mass index (BMI), physical activity and the clinical picture (160, 162). An individual's response to any food and/or food component is assigned to a number of factors including overall state of the health, the genetic profile and physiological environment (163). To minimize side-effects arising from the consumption of physiologically unsuitable food products by an individual, identification of the factors that may predispose the individual to specific diseases as the result of the diet leads to a proposed customized diet. Knowing the sequence of the human genome, it is feasible to develop a personalized diet regime that can be used by each individual based on his/her genetic make-up.

Personalized diet is important for the development of foods that may be used as a "drug" for the prevention of a specific disease affecting that individual. The use of personalized diet will make dietary interventions more efficient by simply changing the diet that have been proven ineffective in certain genotypes (16, 164). The first documented attempt to develop personalized nutrition practices was the ancient medicine system of Ayurveda, that goes back to 1500 B.C.E. Ayurveda as a traditional medicine system, represents a set of comprehensive healthcare practices involving medicine, nutrition, science, and philosophy (165). According to Ayurveda, predisposition to a disease depends on an individual's basic constitution (Prakriti) which requires a certain diet and health practices to avoid (166). With help from modern predictive medicine, Ayurveda's efforts have been directed toward personalized nutrition based on prospective disease and markers for their conditions. Individuals from the three basic constitution types as defined by Prakriti type do show major differences for each type at the genome-wide expression level, as well as their biochemical and hematological parameters including lipid profiles, liver function tests, and hemoglobin content (165). Since the genetic expressions are strongly affected and may be altered by diet, an unhealthy lifestyle and environmental factors, the dietetic principles of Ayurveda have been claimed to help maintain genetic expression (167, 168).

A modern relook into the basics of Ayurveda dietetics had a strong relation to epigenomics, proteomics and metabolomics, which led to the emergence of the concept of personalized nutrition called Ayurnutrigenomics (168). This emerging field of research may show the possibilities of smart dietary choices that will help to prevent non-communicable diseases and lifestyle disorders caused by gene alteration through a fresh insight into specific dietary recommendations based on the genotype of individuals (167–169).

The ability of food components to interfere with molecular mechanisms on a genetic lever has raised consumer interest in considering personalized diet. The consumers are becoming more intent on matching their own genotypes and phenotypes to a diet that will help achieving desired physiological outcomes (16). Personalized diet can, therefore, be viewed as a solution to consumers' needs for health promoting diets and dietary advice (170). However, personalized nutrition needs the development of personalized food products. Working with the variations in individual needs based on biological characteristics of the body, personalized nutrition provides recommendations on types of foods and their optimal intakes. Personalization of food products requires knowledge of their nutrient composition and greater understanding of all possible interactions and impact of micro- and macronutrients on the individual's health (43).

To make personalized diet efficient for an individual, relevant personalized food products must be developed and made available. Current production of personalized products includes not only knowing the genomics of consumers, but also characteristics of nutrients in a food matrix, development of food products with specific functional properties (43), and application of advanced technologies that incorporate elements of Industry 4.0 (30). Therefore, mass production of personalized food products is currently not feasible without wider applications of Industry 4.0 technologies, since it is a comprehensive, laborious, and time-consuming process requiring specific knowledge in the fields of medicine, genomics, nutrition, and food technology. The involvement of Industry 4.0 is required for wider use of the information technologies needed to model and describe the biological processes in medicine and nutrition, including statistics and data processing, genomics, epigenomics, proteomics and metabolomics, which would help to accelerate the development and spread of personalized foods, as well as increase consumer awareness and acceptance of personalized foods. Therefore, personalization will also require consumer input to define food preferences within potential choices (43). Several attempts have been taken to develop personalized foods that could match the genetics of an individual with direct involvement of that consumer. During these trials, European consumers, particularly with health issues, showed an openness and interest in the food personalization research (171). There were also attempts to develop personalized foods by selecting and combining food ingredients in accordance with personal requirements and preferences, thus enabling consumers to contribute to the personalization of food while feeling satisfied and socially involved in the process (172, 173).

Thus, personalization of food is a complex process that requires the development of personalized food products based on the nutritional profile of raw material and genomics of a specific individual, taking into account all the interactions between nutrients and other compounds in the food matrix during processing and storage, and their influence on health and consumer acceptance of the final products.

Fortification and gene editing of foods

Food fortification is a widely used strategy to address the problem of nutrient deficiency and prevent malnutrition. As traditional fortification approaches, i.e., the direct addition of nutrients (e.g., vitamins and minerals) to foods during processing, can be challenged by bioavailability issues, the development biofortification has been accelerated in recent years. The development of fortification techniques, microencapsulation and stabilization technologies, and the intervention of genetic engineering and breeding pave the way to develop various foods (especially staple food crops, such as rice and dairy products, such as cheese) with a higher nutritional value and/or a greater content of health-promoting compounds compared to traditionally fortified foods (174–176).

Genome editing has been applied as an alternative approach to improve the nutrient contents of crops and livestock products. Figure 4 shows the process for the production of cellular meat with its pros and cons. Scientists starting in 2003 have developed gene-editing (GE), which allowed them to develop modified crops and livestock with high performance across a variety of features including both abiotic and biotic stresses (20). GE is the capacity to make exact alterations to a live organism's DNA sequence, thereby modifying its genetic composition. This method works by using enzymes, notably nucleases, designed to target a specific DNA sequence. These enzymes function like scissors, cutting the DNA at a specific spot, and allowing the removal of existing DNA and the insertion of replacement DNA (21). Among the effector nucleases used for GE are meganucleases (MegN), transcription activator-like effector nucleases (TALEN), and zinc-finger nucleases (ZFN). Discovered in 2012 (21), clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) allowed molecular scissors to precisely target a gene in the genome. The procedure first requires identifying a gene responsible for a particular function that requires editing. To edit DNA, a guide RNA (ribonucleic acid) is constructed and the Cas9 enzyme cuts the specified sequence at a designated spot. After the cut, certain functionalities may be added or modified, and the cell can be restored using enzymes.

In an organism, the alterations are accomplished once the guide RNA and Cas9 enzyme are eliminated (177). Overall, GE breeders achieve a particular genotype that occurs naturally at a low frequency, and Cas9 is a crucial differentiator for both breeders looking to establish new lines of animals and regulators who understand that the results are similar to natural mutations. The use of GE methods has opened up many possibilities for generating crops and animals that can better deal with the issues of food and nutrition security. A few examples:

Rice has been a staple for more than half the world's population. Therefore, the first study using CRISPR-Cas9 technology focused on rice GE (178). Drought and salt are two critical abiotic factors that influence rice that GE might address. The use of CRISPR-Cas9 to knockout OsRR22, a gene linked to salt sensitivity in rice increased its success with high saline conditions (0.75% NaCl) without reducing grain production, plant biomass, or grain quality. These GE lines were 19% shorter, whereas wild-type plants were 32% shorter with salt. Likewise, GE plants had no significant changes from the unedited plants in the absence of saline and had considerably less severe biomass decreases due to salt exposure. Saline tests were done in greenhouses, and overall agronomic performance was assessed in the field. Compared to wild-type plants, GE plants had substantially less severe biomass losses due to salt exposure (179). Other rice editing efforts have resulted in early maturing rice that is more suitable for cultivation in the northern hemisphere, where it needs a longer growing period and colder temperatures (180). Rice plants were GE using CRISPR-Cas9 to target the flowering-related genes Hd2,4, and 5, resulting in plants that bloomed considerably quicker than their wild-type counterparts.

In the future, when temperatures and other climatic conditions in tropical areas make agriculture less productive, early flowering plants may be better suited. Aside from being adapted to water shortages, the early blooming may also reduce the amount of cumulative water needed to grow to harvest. GE technologies may assist with knock-ins as well as knockouts. To improve drought tolerance, researchers used CRISPR-Cas9 to place a promoter into a particular maize gene. An alternative maize promoter was placed before ARGOS8, a drought-tolerant gene. This precise insertion resulted in higher grain yields with water stress during flowering while preserving normal growth conditions. This method is an intragenic strategy using GE in which a native maize genetic sequence was placed at a new locus to improve plant tolerance to the abiotic stressor (181).

The development of disease resistance in pigs has also benefited from GE. Two genes, CD163 and CD1D were knocked out (182). The former is essential for PRRS viral infection, whereas the latter is involved in innate immunity. The Cd163 knockout pigs were tested for PRRS resistance and had no symptoms when infected. On the other hand, the wild-type offspring had severe symptoms and had to be euthanized (182). By using CRISPR-Cas9 GE to knock out CD163, pigs might become immune to PRRSV (183). CRISPR-Cas9 has been used to knock-in resistance to the classical swine fever virus (CSFV) at the Rosa26 gene. This locus is a suitable target for transgenic insertion because of its widespread and high expression, and

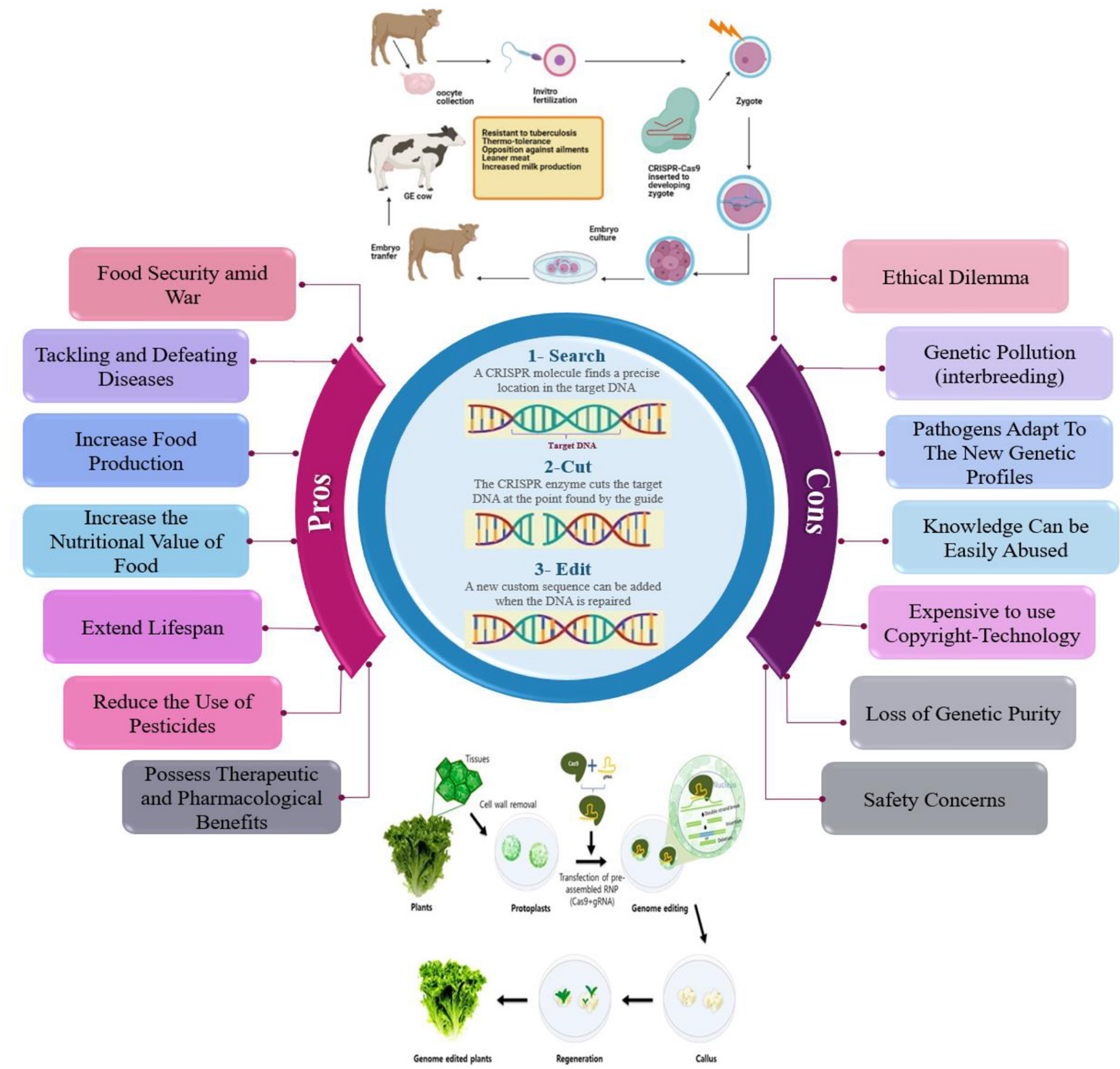

FIGURE 4
Process for the gene-editing with its pros and cons.

the absence of any gene-silencing effects (184). The gene-edited pigs were CSFV-resistant, but all wild-type pigs died each time. The *C. elegans* fat-1 gene was inserted into the Rosa26 locus in pigs, providing a proof-of-concept to illustrate the feasibility of concurrently boosting the nutritional value of pork while raising general disease resistance since fat-1 is involved in both disease resistance and the nutritive quality of meat (184). Also, the ANPEP (aminopeptidase N) gene was knocked out using CRISPR-Cas9 GE to impart resistance to coronavirus infections (185).

Consumers' acceptance or rejection of food produced from GE crops may have significant economic consequences at all stages of the food system. Global agreement on the safety and regulation of GE crops is non-existent, and this is a serious and significant problem. GE in food production is accepted by many nations and areas, while Europe and New Zealand have adopted a more cautious approach (186). In a study of around 10,000 individuals done by the University of Tokyo, 40 to 50% of respondents said they did not want to eat GE crops or animal products (186). Another study investigated people's attitudes toward GE in food plants and animals. People tended to show more positive attitudes toward GE plants than GE animals. Their acceptance was stronger for biotechnology medical applications than agri-food applications (44). Consumers have

TABLE 2 New food production systems.

Type of agriculture technique	Definition	Advantages	Disadvantages
Hydroponic	Cultivation of plants without soil using mineral nutrient solutions in aqueous solvents	No soil involved and no need for soil preparing or testing Optimal use of location (20% less space for growing) Complete control over climate Conserving water by reusing it (20% less water than traditional agriculture) Optimal use of nutrients Zero weeding, mulching, etc. Faster growth rate Total control over the nutritional balance Fewer pests and ailments since the environment is sterile	Constant monitoring is required Water-based microorganisms may readily infiltrate the system All plants in the system will be impacted if a disease emerges If the system fails without soil to act as a buffer, plant death will ensue quickly Risks of water and electricity failure Requires a high level of technical knowledge Some plants are difficult to grow hydroponically (such as potatoes) Could be expensive
Aquaponics	A food production system integrating aquaculture and hydroponics to grow fish and plants together in one system	Efficient use of water and nutrients Organic fertilization Environmentally friendly Produce the highest yield on a field Smart vertical farming Consistent with circular agriculture	Limited crops High initial cost High consumption of electricity Unsustainable fish food The system must be professionally installed Unexpected failure
Aeroponics	A method of growing plants without any growing medium where the roots are suspended in the air, and nutrient solutions are delivered to the plant using a fine mist or spray	Completely controlled environment Uses fewer resources, e.g., 90% less water than traditional farming Saves considerable space and soil Fast growth and high yield As roots grow in the air, there is no physical medium inhibiting a plant's expansion Growth environment can be pest- and disease-free	Requires advanced machinery and equipment to operate Expensive initial investment Dependency on electricity Requires more monitoring and maintenance compared to the other systems Nutrient content of the solution must be monitored carefully and constantly Extremely sensitive system

historically reacted negatively to genetically modified products (GMO) because of their perceived "unnaturalness," and GE foods may encounter the same problems. Food that is more nutrient-dense, environmentally and animal welfare-friendly and cost-effective may be created by GE and overtime become consumer acceptable.

GE discussions need to be framed so as to enable the public to participate in the discussion, manage any misunderstandings, and maintain consumer trust. GE products may also need labeling that is clear and accurate.

Hydroponics, aquaponics, and other indoor vertical food production systems

Agriculture-based food production growth is now much lower than the rate of population expansion, which is a concern. As a result, more agricultural production systems must be implemented to improve and achieve expected future food supply needs. Alternative forms of farming systems have become more popular. Hydroponics, aquaponics, and other indoor vertical farming systems are some of the primary sectors where global agricultural output may be improved as growing conditions can be better managed. Table 2 shows the pros and cons of hydroponics, aquaponics, and aeroponics systems. Hydroponics is a type of horticulture and a subset of hydroculture that includes mineral fertilizer solutions in an aqueous solvent to grow plants, mainly crops, without soil (187). Any crop may be grown hydroponically, but the most frequent are leaf lettuce, celery, cucumbers, peppers, tomatoes, strawberries, watercress, and various herbs (24). Aquaponics is an indoor vertical farming system combining aquaculture (fish farming) and hydroponics. In aquaponics, farmed fish waste provides nutrients for hydroponically grown plants, which in turn clean the water for the fish. This ensures a closed-loop, long-term feeding supply. Because few pesticides and herbicides are non-toxic to fish, aquaponics production relies on organic pest and weed management (22, 188, 189). Aeroponics is a soilless revolutionary farming system that allows growing plants in the air, where plants' roots are suspended in a mist of nutrient solution (49, 190). This system is well suitable to automation, digitalization, and other advanced technologies. For example, an automated IoT-based aeroponics system, with remote data monitoring, including sensors measuring temperature, humidity, pH value of the water, and the light exposure, has been developed (23).

Integration of Industry 4.0 technologies to food production systems is termed smart farming or precision farming (190–192). Application of Industry 4.0 innovations (e.g., robotics, IoT, drones, satellite imagery, and smart sensors) in food production systems can improve productivity and enable data collection and aggregation, providing further improvements of precision technology and possible solutions to various problems, which could not be solved with traditional farming systems (24, 191, 192). A few examples:

The application of drones is now being investigated across various production sectors, including agricultural supply chains, providing relevant opportunities to overcome challenges (193). The application of Industry 4.0 technologies to aquaponics is termed Aquaponics 4.0, referring to a digital aquaponics farm that involves remote monitoring and control of ecosystem parameters, a high degree of automation, and intelligent decision-making to ensure high crop yields and quality (22, 188).

Despite the global spread of these alternative farming systems, only a limited number of studies discuss the overall health of the plants grown using hydroponics, aquaponics, and aeroponics, and the consumer acceptance of these foods. A study of hydroponics and aquaponics cultivation showed that >60% of consumers are generally unfamiliar with these systems and their products (194, 195). Overall, three major categories of features allow classifying consumers' attitudes and beliefs, and identifying prospective buyers of these farming products. These factors include (i) the consumers' personal and sociodemographic characteristics and their prior knowledge; (ii) the consumers' willingness to pay some percentage for locally produced or pesticide/herbicide and antibiotic-free products before the concept of these cultivations was introduced; and (iii) the consumers' willingness to pay some percentage for products after the concept of these systems was introduced and it meets consumers' main values regarding those products.

Conclusions

The potential of the Industry 4.0 revolution technologies to enhance eight food trends (namely, plant-based, insect-based, cell-based, 3D-printed, personalized, and GE foods, as well as foods resulting from by-products and ugly produce, and new production systems) were explored in this narrative review. According to the Scopus database, there has been a significant increase in the number of publications and citation on these food trends. The role of emerging technologies in promoting more acceptability and consumption of proteins from these traditional (such as plant- and insect-based foods) and innovative (such as GE foods, cell-based meat, and 3D printed products) alternative sources has been highlighted. The main outcome of this review paper is to broaden readers understanding of the applicability of emerging and innovative techniques to achieve a shift to digital and ecological transitions toward greener, healthier, and more sustainable diets.

The first, second, and third industrial revolutions were characterized by mechanization, electrification, and information advances, respectively, while automation, digitalization, hyperconnectivity, as well as fusion between physical, digital, and biological worlds are the main features of the ongoing fourth industrial revolution, called Industry 4.0. Industry 4.0 is a huge umbrella term that includes artificial intelligence, big data, smart sensors, robotics, and block chain, to name a few.

Traditional food production systems, such as livestock farming, have been identified as a contributor to climate change and unsustainable development. There is therefore a search for alternative proteins that have comparable health and sensory characteristics to traditional animal-based products but with a lower environmental footprint. PB sources have traditionally been the most investigated, especially PB proteins from oilseeds (e.g., rapeseed and hemp), legumes (e.g., lentils, beans, and peas), and cereals (e.g., wheat and rice), as well as fruits and vegetables for use as food and feed. Although in use since antiquity, an increased consumer interest in these foods has recently emerged with a growing number of vegans, vegetarians, and flexitarians. In addition, a variety of PB meat, fish, milk, and egg analogs have been seen as a promising sustainable approach to reducing the consumption of meat and other animal-based proteins (10, 34, 35). A challenge to consumption of PB foods is many of their poor nutritional and functional properties. However, this issue can be solved by blending different types of proteins from various sources and optimizing processing conditions, thus improving protein quality, digestibility, and bioavailability (32, 33). Additionally, current research shows that Industry 4.0 innovations and emerging processing technologies could help to improve their nutritional and technological functionality as well as their sensory perception. The recent innovations and advancements could be a major driver in convincing consumers to rely more on PB diets.

The popularity of insect-based diets has been increasing spurred by the increased awareness and demand of consumers for sustainable alternatives to animal proteins. Insects could be the food of the future, but currently most consumers do not seem willing to adopt the consumption of insect proteins. Therefore, continued efforts will be required to change the minds and behaviors of consumers. Industry 4.0 technologies and new innovations in processing technologies and analytical approaches such as metabolomics (36) could help to improve the sensory and techno-functional properties as well as digestibility of insect-based foods, enhancing their acceptability and making them more appealing to consumers. Many insect-based products (e.g., insect flour, insect protein powders, and insect protein hydrolysates) can be prepared from numerous edible insect species (especially crickets and mealworms) and can be used as snacks or ingredients to produce other food or feed. Insect foods are nutritious, cheap, and sustainable as less food, land, and water are required for insect breeding than raising cattle or other livestock. Although positive aspects of insect proteins regarding both nutritional and environmental issues has decreased entomophobia, intensive studies on safety, hygiene and toxicity, marketing strategies, and governmental regulations should be done to accelerate consumer acceptance of these alternative portion sources.

Cell-cultured meat and related products (e.g., seafood and poultry) seem to be one of the most promising and revolutionary strategies to achieve environmental sustainability and improve animal welfare, hence the large number of patents and publications (13). As the process of production occurs in the laboratory, water and land requirements as well as greenhouse gas emissions are low. Although the technique has the potential to disrupt and transform the whole agricultural and food industry, it is still costly and production at a large scale has not yet been implemented. Moreover, concerns about the naturalness, ethical issues, and safety of cultured meat and related products still exist among consumers, while studies on health benefits, funding resources and appropriate regulatory pathways are still required (14, 196).

3D printing has been accepted by many scientific and industrial areas, including the food industry. This technique could enable producing on-demand, complex, and customized (e.g., shapes, sizes, tastes, texture, and nutritional properties) food products to satisfy a range of consumer preferences. Several 3D printing techniques have been developed, with extrusion-based printing being the most common. The last few years have seen significant progress in this field, with the emergence of new smart materials, new technologies, and significant innovations, accelerating the move toward more advanced and innovative additive manufacturing technologies, including 4D, 5D, and 6D printing (40, 41). Although the scope of current application is limited to the decoration and fabrication of a few food products such as chocolates, cookies, and cakes (39), further improvement in functional and nutritional properties of printed foods is expected with the advent of Industry 4.0 innovations, enhancing consumer acceptance. Owning a personal food printer at home is probably likely in the not so distant future. One of the possible applications of food printing is personalized foods (or personalized nutrition) as food can be specifically printed to meet an individual's health-nutritional needs, including medicinal and nourishment requirements (16, 17).

Industry 4.0 technologies should be considered to reduce the environmental impact of food production systems and achieve zero-waste. According to FAO, a huge amount of food by-products and ugly produce are wasted or lost every day. Increased concerns about environmental sustainability are driving the growing interest in better uses of food waste and by-products, and ugly produce. Technological innovations and scientific advances along with education could help consumers accept the hidden beauty of ugly food, thus reducing food waste and contributing to food sustainability.

Gene editing methods have opened up many possibilities for generating crops and animals with desired traits. New gene editing tools (e.g., CRISPR-Cas9-based GE) are being rapidly developed, taking advantages of recent progress in genetic engineering. GE is efficient and can enhance product quality and increase resistance against diseases and pests with a low risk of off-target effects. GE could bring about new possibilities in agriculture and biomedicine but consumer acceptance of GE products is still low. High-productivity food systems, including hydroponics, aquaponics, and other indoor vertical farming methods, are occurring in smart controlled food production environments. These smart or precision farming methods are becoming better understood to fulfill future food demands.

Future perspectives

While this review is not an exhaustive overview of all emerging food trends, eight of the more pertinent ones, from food technological advances perspectives, were discussed. Each of these emerging food trends has been fostered by the greater use of Industry 4.0 technologies and recent advances in many fields of food science and technology. Innovative solutions based on Industry 4.0 enablers (such as AI, smart sensors, and robotics) can be used to increase agriculture productivity, optimize production conditions, and reduce waste and loss, accelerating the green and digital transition of future food production systems. The interest in traditional animal-proteins alternatives, including plant-based foods and insects and more recent food trends, such as cell-cultured meat, 3D-printed, fortified, and gene-edited foods are likely to continue growing in popularity in response to the increasing consumers' awareness regarding the environmental impact of food choices. With the ongoing rapid technological advances in physical, biological, and digital worlds, other food trends are expected to emerge in the future.

Although the concept of Industry 4.0 may have previously had greater significance to other industries, the opportunities for the agriculture and food industry sectors are also enormous. Wider applications of Industry 4.0 technologies in the agriculture and food industry could enable the production of foods with higher quality and affordability, and lower environmental impact. Innovative technologies provide opportunities for improving sensory and nutritional properties of foods, thus increasing their positive perception by consumers, which in turn could enhance food sustainability and contribute to addressing the issue of global food insecurity.

Author contributions

Conceptualization, methodology, and writing—original draft preparation: AH. Writing—original draft preparation: JC, MT, AVR, OB, GN, YJ, FS, MA, PM, and AK. Writing—review and editing: AH and JR. Funding acquisition: JC. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author MT was employed by Centre for Innovative Process Engineering (CENTIV) GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Bisoffi S, Ahrné L, Aschemann-Witzel J, Báldi A, Cuhls K, DeClerck F, et al. COVID-19 and sustainable food systems: what should we learn before the next emergency. *Front Sustain Food Syst.* (2021) 5:53. doi: 10.3389/fsufs.2021.650987

2. Galanakis CM, Rizou M, Aldawoud TMS, Ucak I, Rowan NJ. Innovations and technology disruptions in the food sector within the COVID-19 pandemic and post-lockdown era. *Trends Food Sci Technol.* (2021) 110:193–200. doi: 10.1016/j.tifs.2021.02.002

3. Janssen M, Chang BPI, Hristov H, Pravst I, Profeta A, Millard J, et al. Changes in food consumption during the COVID-19 pandemic: analysis of consumer survey data from the first lockdown period in Denmark, Germany, and Slovenia. *Front Nutr.* (2021) 8:1–20. doi: 10.3389/fnut.2021.635859

4. Liu F, Li M, Wang Q, Yan J, Han S, Ma C, et al. Future foods: Alternative proteins, food architecture, sustainable packaging, and precision nutrition. *Crit Rev Food Sci Nutr.* (2022) 2022:1–22. doi: 10.1080/10408398.2022.2033683

5. Anzani C, Boukid F, Drummond L, Mullen AM, Álvarez C. Optimising the use of proteins from rich meat co-products and non-meat alternatives: nutritional, technological and allergenicity challenges. *Food Res Int.* (2020) 137:109575. doi: 10.1016/j.foodres.2020.109575

6. Onwezen MC, Bouwman EP, Reinders MJ, Dagevos HA. systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. *Appetite.* (2021) 159:105058. doi: 10.1016/j.appet.2020.105058

7. Valoppi F, Agustin M, Abik F, Carvalho DMD, Sithole J, Bhattarai M, et al. Insight on current advances in food science and technology for feeding the world population. *Front. Sustain. Food Syst.* (2021) 5:1–17. doi: 10.3389/fsufs.2021.626227

8. Kurek MA, Onopiuk A. Pogorzelska-nowicka, E, Szpicer, A, Zalewska, M. Novel protein sources for applications in meat-alternative products — insight and challenges. *Foods.* (2022) 11:957. doi: 10.3390/foods11070957

9. Sim SYJ, Srv A, Chiang JH, Henry CJ. Plant proteins for future foods: a roadmap. *Foods.* (2021) 10:1967. doi: 10.3390/foods10081967

10. McClements DJ, Grossmann L. The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Compr Rev Food Sci Food Saf.* (2021) 20:4049–100. doi: 10.1111/1541-4337.12771

11. Mancini S, Sogari G, Diaz SE, Menozzi D, Paci G, Moruzzo R, et al. Exploring the future of edible insects in Europe. *Foods.* (2022) 11:455. doi: 10.3390/foods11030455

12. Smith MR, Stull VJ, Patz JA, Myers SS. Nutritional and environmental benefits of increasing insect consumption in Africa and Asia. *Environ. Res. Lett.* (2021) 16. doi: 10.1088/1748-9326/abf06c

13. Ng ET, Singh S, Yap WS, Tay SH, Choudhury D. Cultured meat - A patentometric analysis. *Crit Rev Food Sci Nutr.* (2021) 2021. doi: 10.1080/10408398.2021.1980760

14. Chriki S, Hocquette JF. The myth of cultured meat: A review. *Front Nutr.* (2020) 7:7. doi: 10.3389/fnut.2020.00007

15. Singh S, Yap WS, Ge XY, Min VLX, Choudhury D. Cultured meat production fuelled by fermentation. *Trends Food Sci Technol.* (2022) 120:48–58. doi: 10.1016/j.tifs.2021.12.028

16. Derossi A, Husain A, Caporizzi R, Severini C. Manufacturing personalized food for people uniqueness. An overview from traditional to emerging technologies. *Crit Rev Food Sci Nutr.* (2020) 60:1141–59. doi: 10.1080/10408398.2018.1559796

17. Escalante-Aburto A, Santiago GT, Álvarez MM, Chuck-Hernández C. Advances and prospective applications of 3D food printing for health improvement and personalized nutrition. *Compr Rev Food Sci Food Saf.* (2021) 2021. doi: 10.1111/1541-4337.12849

18. Handral K, Hua Tay H, Wan Chan S, Choudhury WD. 3D Printing of cultured meat products. *Crit Rev Food Sci Nutr.* (2022) 62:272–81. doi: 10.1080/10408398.2020.1815172

19. Castro-Muñoz R, Díaz-Montes E, Gontarek-Castro E, Boczkaj G, Galanakis CMA. comprehensive review on current and emerging technologies toward the valorization of bio-based wastes and by products from foods. *Compr Rev Food Sci Food Saf.* (2022) 21:46–105. doi: 10.1111/1541-4337.12894

20. Arshad RN, Abdul-Malek Z, Roobab U, Qureshi MI, Khan N, Ahmad MH, et al. Effective valorization of food wastes and by-products through pulsed electric field: a systematic review. *J Food Process Eng.* (2021) 44:1–14. doi: 10.1111/jfpe.13629

21. Ulug SK, Jahandideh F, Wu J. Novel technologies for the production of bioactive peptides. *Trends Food Sci Technol.* (2021) 108:27–39. doi: 10.1016/j.tifs.2020.12.002

22. Kumar A, Anju T, Kumar S, Chhapekar SS, Sreedharan S, Singh S, et al. Integrating omics and gene editing tools for rapid improvement of traditional food plants for diversified and sustainable food security. *Int J Mol Sci.* (2021) 22:8093. doi: 10.3390/ijms22158093

23. Khalil AM. The genome editing revolution: a review. *J Genet Eng Biotechnol.* (2020) 18:1–16. doi: 10.1186/s43141-020-00078-y

24. Karavolias NG, Horner W, Abugu MN, Evanega SN. Application of gene editing for climate change in agriculture. *Front Sustain Food Syst.* (2021) 5:296. doi: 10.3389/fsufs.2021.685801

25. Abbasi R, Martinez P, Ahmad R. An ontology model to represent aquaponics 4, 0 system's knowledge. *Inf Process Agric.* (2021). doi: 10.1016/j.inpa.2021.12.001 (In press).

26. Francis F, Vishnu PL, Jha M, Rajaram B. IOT-based automated aeroponics system. *Lect Notes Electr Eng.* (2018) 492:337–45. doi: 10.1007/978-981-10-8575-8_32

27. Hemathilake DMKS, Gunathilake DMCC. High-productive agricultural technologies to fulfill future food demands: Hydroponics, aquaponics, and precision/smart agriculture. In: Bhat R, Ed. *Future Foods: Global trends, opportunities, and sustainability challenges*. London, UK: Academic Press. (2022) (2022):555–67. doi: 10.1016/B978-0-323-91001-9.00018-9

28. Sadeghi K, Kim J, Seo J. Packaging 4, 0 The threshold of an intelligent approach. *Compr Rev Food Sci Food Saf.* (2022) 21:2615–38. doi: 10.1111/1541-4337.12932

29. Nosalska K, Piatek ZM, Mazurek G, Rzadca R. Industry 4.0: Coherent definition framework with technological and organizational interdependencies. *J Manuf Technol Manag.* (2020) 31:837–62. doi: 10.1108/JMTM-08-2018-0238

30. Hassoun A, Aït-Kaddour A, Abu-Mahfouz AM, Rathod NB, Bader F, Barba FJ, et al. The fourth industrial revolution in the food industry—part I: Industry 4.0 technologies. *Crit. Rev. Food Sci. Nutr.* (2022) 0:1–17. doi: 10.1080/10408398.2022.2034735

31. Hassoun A, Siddiqui SA, Smaoui S, Uca, I., Arshad, R. N., Garcia-Oliveira, P., et al. (2022). Seafood processing, preservation, and analytical techniques in the age of Industry 4.0. Appl. Sci. 1703, 12. doi: 10.3390/app12031703

32. Jiménez-Munoz LM, Tavares GM, Corredig M. Design future foods using plant protein blends for best nutritional and technological functionality. *Trends Food Sci Technol.* (2021) 113:139–50. doi: 10.1016/j.tifs.2021.04.049

33. Boukid F, Rosell CM, Castellari M. Pea protein ingredients: A mainstream ingredient to (re)formulate innovative foods and beverages. *Trends Food Sci Technol.* (2021) 110:729–42. doi: 10.1016/j.tifs.2021.02.040

34. McClements DJ, Grossmann LA. brief review of the science behind the design of healthy and sustainable plant-based foods. *NPJ Sci Food.* (2021) 5:1–10. doi: 10.1038/s41538-021-00099-y

35. Grossmann L, McClements DJ. The science of plant-based foods: Approaches to create nutritious and sustainable plant-based cheese analogs. *Trends Food Sci Technol.* (2021) 118:207–29. doi: 10.1016/j.tifs.2021.10.004

36. Poma G, Cuykx M, Silva Da, Iturrospe KM, van Nuij, E, et al. (2022). The metabolomics era. First steps towards the implementation of entometabolomics in food systems. *Trends Food Sci. Technol.* 119:371–7. doi: 10.1016/j.tifs.2021.12.018

37. Acosta-Estrada BA, Reyes A, Rosell CM, Rodrigo D, Ibarra-Herrera CC. Benefits and challenges in the incorporation of insects in food products. *Front Nutr.* (2021) 8:344. doi: 10.3389/fnut.2021.687712

38. Liceaga AM. Processing insects for use in the food and feed industry. *Curr Opin Insect Sci.* (2021) 48:32–6. doi: 10.1016/j.cois.2021.08.002

39. Singhal S, Rasane P, Kaur S, Garba U, Bankar A, Singh J, et al. 3D food printing: Paving way towards novel foods. *An Acad Bras Cienc.* (2020) 92:1–26. doi: 10.1590/0001-3765202020180737

40. Oral MO, Derossi A, Caporizzi R, Severini C. Analyzing the most promising innovations in food printing. Programmable food texture and 4D foods. *Futur Foods.* (2021) 4:100093. doi: 10.1016/j.fufo.2021.100093

41. Ghazal AF, Zhang M, Mujumdar AS, Ghamry M. Progress in 4D/5D/6D printing of foods: applications and R&D opportunities. *Crit Rev Food Sci Nutr.* (2022) 1–24. doi: 10.1080/10408398.2022.2045896

42. Szakaly Z, Feher A, Kiss M. Consumer acceptance of personalized nutrition. In *Trends in personalized nutrition*. Galanakis, C.M., Ed. London, UK: Academic Press. (2019) p. 225–260. doi: 10.1016/B978-0-12-816403-7.00009-X

43. Ueland Ø, Altintzoglou T, Kirkhus B, Lindberg D, Rognså GH, Rosnes JT, et al. Perspectives on personalised food. *Trends Food Sci Technol.* (2020) 102:169–77. doi: 10.1016/j.tifs.2020.05.021

44. Kato-Nitta N, Inagaki Y, Maeda T, Tachikawa M. Effects of information on consumer attitudes towards gene-edited foods: a comparison between livestock and vegetables. *CABI Agric Biosci.* (2021) 2:1–12. doi: 10.1186/s43170-021-00029-8

45. Chamorro F, Carpena M, Fraga-Corral M, Echave J, Riaz Rajoka MS, Barba FJ, et al. Valorization of kiwi agricultural waste and industry by-products by recovering bioactive compounds and applications as food additives: A circular economy model. *Food Chem.* (2022) 370:131315. doi: 10.1016/j.foodchem.2021.131315

46. Ozogul F, Cagalj M, Šimat V, Ozogul Y, Tkaczewska J, Hassoun A, et al. Recent developments in valorisation of bioactive ingredients in discard/seafood processing by-products. *Trends Food Sci Technol.* (2021) 116:559–82. doi: 10.1016/j.tifs.2021.08.007

47. Socas-Rodríguez B, Álvarez-Rivera G, Valdés A, Ibáñez E, Cifuentes A. Food by-products and food wastes: are they safe enough for their valorization? Trends Food Sci. *Technol.* (2021) 114:133–47. doi: 10.1016/j.tifs.2021.05.002

48. Hartmann T, Jahnke B, Hamm U. Making ugly food beautiful: consumer barriers to purchase and marketing options for Suboptimal Food at retail level – A systematic review. *Food Qual Prefer.* (2021) 90:104179. doi: 10.1016/j.foodqual.2021.104179

49. Lakhiar IA, Gao J, Syed TN, Ali Chandio F, Tunio MH, Ahmad F, et al. Overview of the aeroponic agriculture – An emerging technology for global food security. *Int J Agric Biol Eng.* (2020) 13:1–10. doi: 10.25165/j.ijabe.20201301.5156

50. Amorim d, Borchardt Deggau WS. do Livramento Gonçalves G, da Silva Neiva S, Prasath AR, Salgueirinho Osório de Andrade Guerra JB. Urban challenges and opportunities to promote sustainable food security through smart cities and the 4th industrial revolution. Land use policy. (2019) 87:104065. doi: 10.1016/j.landusepol.2019.104065

51. Jambrak AR, Nutrizio M, Djekić I, Pleslić S, Chemat F. Internet of nonthermal food processing technologies (Iontp): Food industry 4.0 and sustainability. *Appl Sci.* (2021) 11:1–20. doi: 10.3390/app11020686

52. Bigliardi B. Industry 4.0 applied to food. In: *Sustainable food processing and engineering challenges*. Galanakis CM, Ed. London, UK: Academic Press. (2021) p. 1–23. doi: 10.1016/B978-0-12-822714-5.00001-2

53. Khan N, Ray RL, Kassem HS, Hussain S, Zhang S, Khayyam M, et al. Potential role of technology innovation in transformation of sustainable food systems: a review. *Agric.* (2021) 11:984. doi: 10.3390/agriculture11100984

54. Kakani V, Nguyen VH, Kumar BP, Kim H, Pasupuleti VR. A critical review on computer vision and artificial intelligence in food industry. *J Agric Food Res.* (2020) 2:100033. doi: 10.1016/j.jafr.2020.100033

55. Mavani NR, Ali JM, Othman S, Hussain MA, Hashim H, Rahman NA, et al. Application of artificial intelligence in food industry — a guideline. *Food Eng Rev.* (2022) 2022:134–75. doi: 10.1007/s12393-021-09290-z

56. Chakka AK, Sriraksha MS, Ravishankar CN. Sustainability of emerging green non-thermal technologies in the food industry with food safety perspective: a review. *LWT.* (2021) 151:112140. doi: 10.1016/j.lwt.2021.112140

57. Zhang ZH, Wang LH, Zeng XA, Han Z, Brennan CS. Non-thermal technologies and its current and future application in the food industry: a review. *Int J Food Sci Technol.* (2019) 54:1–13. doi: 10.1111/ijfs.13903

58. Misra NN, Dixit Y, Al-Mallahi A, Bhullar MS, Upadhyay R, Martynenko A, et al. IoT, big data and artificial intelligence in agriculture and food industry. *IEEE Internet Things J.* (2022) 2020:1–1. doi: 10.1109/JIOT.2020.2998584

59. Astill J, Dara RA, Campbell M, Farber M, Fraser EDG, Sharif S, et al. Transparency in food supply chains : A review of enabling technology solutions. *Trends Food Sci Technol.* (2019) 91:240–7. doi: 10.1016/j.tifs.2019.07.024

60. Popa A, Hnatiuc M, Paun M, Geman O, Hemanth DJ, Dorcea D, et al. An intelligent IoT-based food quality monitoring approach using low-cost sensors. *Symmetry.* (2019) 11:374. doi: 10.3390/sym11030374

61. Aamer AM, Al-Awlaqi MA, Affia I, Arumsari S, Mandahawi N. The internet of things in the food supply chain: Adoption challenges. *Benchmarking.* (2021) 28:2521–41. doi: 10.1108/BIJ-07-2020-0371

62. Onwude DI, Chen G, Eke-Emezie N, Kabutey A, Khaled AY, Sturm B, et al. Recent advances in reducing food losses in the supply chain of fresh agricultural produce. *Processes.* (2020) 8:1–31. doi: 10.3390/pr8111431

63. Poore J, Nemecek T. Reducing food's environmental impacts through producers and consumers. *Science.* (2018) 360:987–92. doi: 10.1126/science.aaq0216

64. Springmann M, Clark M, Mason-D'Croz D, Wiebe K, Bodirsky BL, Lassaletta L, et al. Options for keeping the food system within environmental limits. *Nature.* (2018) 562:519–25. doi: 10.1038/s41586-018-0594-0

65. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the anthropocene: The eat–lancet commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4

66. Alcorta A, Porta A, Tárrega A, Alvarez MD, Vaquero MP. Foods for plant-based diets: Challenges and innovations. *Foods.* (2021) 10:293. doi: 10.3390/foods10020293

67. Tso R, Forde CG. Unintended consequences: Nutritional impact and potential pitfalls of switching from animal- to plant-based foods. *Nutrients.* (2527) (2021):13. doi: 10.3390/nu13082527

68. Kumar A, Mosa KA, Ji L, Kage U, Dhokane D, Karre S, et al. Metabolomics-assisted biotechnological interventions for developing plant-based functional foods and nutraceuticals. *Crit Rev Food Sci Nutr.* (2018) 58:1791–807. doi: 10.1080/10408398.2017.1285752

69. Munekata PES, Domínguez R, Budaraju S, Roselló-Soto E, Barba FJ, Mallikarjunan K, et al. Effect of innovative food processing technologies on the physicochemical and nutritional properties and quality of non-dairy plant-based beverages. *Foods.* (2020) 9:1–16. doi: 10.3390/foods9030288

70. Yu E, Malik VS, Hu FB. Cardiovascular disease prevention by diet modification: JACC health promotion series. *J Am Coll Cardiol.* (2018) 72:914–26. doi: 10.1016/j.jacc.2018.02.085

71. Frank B, Hu M, Otis BO, McCarthy G. Can plant-based meat alternatives be part of a healthy and sustainable diet? *JAMA.* (2019) 322:1547–8. doi: 10.1001/jama.2019.13187

72. Godfray HCJ, Aveyard P, Garnett T, Hall JW, Key TJ, Lorimer J, et al. Meat consumption, health, and the environment. *Science.* (2018) (2018):361. doi: 10.1126/science.aam5324

73. Silva ARA, Silva MMN, Ribeiro BD. Health issues and technological aspects of plant-based alternative milk. *Food Res Int.* (2020) 131:108972. doi: 10.1016/j.foodres.2019.108972

74. Neff RA, Edwards D, Palmer A, Ramsing R, Righter A, Wolfson J, et al. Reducing meat consumption in the USA: a nationally representative survey of attitudes and behaviours. *Public Health Nutr.* (2018) 21:1835–44. doi: 10.1017/S1368980017004190

75. Aschemann-Witzel J, Gantriis RF, Fraga P, Perez-Cueto FJA. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Crit Rev Food Sci Nutr.* (2020) 61:1–10. doi: 10.1080/10408398.2020.1793730

76. Jeske S, Zannini E, Arendt EK. Past, present and future: The strength of plant-based dairy substitutes based on gluten-free raw materials. *Food Res Int.* (2018) 110:42–51. doi: 10.1016/j.foodres.2017.03.045

77. López-Gámez G, Elez-Martínez P, Martín-Belloso O, Soliva-Fortuny R. Recent Advances toward the application of non-thermal technologies in food processing: an insight on the bioaccessibility of health-related constituents in plant-based products. *Foods.* (2021) 1538:10. doi: 10.3390/foods10071538

78. Pham ND, Khan IH, Joardder MUH, Rahman MM, Abesinghe AMN, Karim MA, et al. Quality of plant-based food materials and its prediction during intermittent drying. *Crit Rev Food Sci Nutr.* (2019) 59:1–15. doi: 10.1080/10408398.2017.1399103

79. Le-Bail A, Maniglia BC, Le-Bail P. Recent advances and future perspective in additive manufacturing of foods based on 3D printing. *Curr Opin Food Sci.* (2020) 35:54–64. doi: 10.1016/j.cofs.2020.01.009

80. Testa M, Stillo M, Maffei G, Andriolo V, Gardois P, Zotti CM, et al. Ugly but tasty: A systematic review of possible human and animal health risks related to entomophagy. *Crit Rev Food Sci Nutr.* (2017) 57:3747–59. doi: 10.1080/10408398.2016.1162766

81. House J. Consumer acceptance of insect-based foods in the Netherlands: Academic and commercial implications. *Appetite.* (2016) 107:47–58. doi: 10.1016/j.appet.2016.07.023

82. Halloran A, Roos N, Eilenberg J, Cerutti A, Bruun S. Life cycle assessment of edible insects for food protein: a review. *Agron Sustain Dev.* (2016) 36:1–13. doi: 10.1007/s13593-016-0392-8

83. Orsi L, Voege LL, Stranieri S. Eating edible insects as sustainable food? Exploring the determinants of consumer acceptance in Germany. *Food Res Int.* (2019) 125:108573. doi: 10.1016/j.foodres.2019.108573

84. Elhassan M, Wendin K, Olsson V, Langton M. Quality aspects of insects as food—nutritional, sensory, and related concepts. *Foods.* (2019) 8:95. doi: 10.3390/foods8030095

85. Van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, et al. *Edible insects: Future prospects for food and feed security.* Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). (2013).

86. Duda A, Adamczak J, Chelminska P, Juszkiewicz J, Kowalczewski P. Quality and nutritional/textural properties of durum wheat pasta enriched with cricket powder. *Foods.* (2019) 8:46. doi: 10.3390/foods8020046

87. Rubio NR, Fish KD, Trimmer BA, Kaplan DL. Possibilities for engineered insect tissue as a food source. *Front Sustain Food Syst.* (2019) 3:24. doi: 10.3389/fsufs.2019.00024

88. Wendin KM, Nyberg ME. Factors influencing consumer perception and acceptability of insect-based foods. *Curr Opin Food Sci.* (2021) 40:67–71. doi: 10.1016/j.cofs.2021.01.007

89. Mishyna M, Chen J, Benjamin O. Sensory attributes of edible insects and insect-based foods – Future outlooks for enhancing consumer appeal. *Trends Food Sci Technol.* (2020) 95:141–8. doi: 10.1016/j.tifs.2019.11.016

90. Hartmann C, Shi J, Giusto A, Siegrist M. The psychology of eating insects: a cross-cultural comparison between Germany and China. *Food Qual Prefer.* (2015) 44:148–56. doi: 10.1016/j.foodqual.2015.04.013

91. Motoki K, Ishikawa S, Spence C, Velasco C. Contextual acceptance of insect-based foods. *Food Qual Prefer.* (2020) 85:103982. doi: 10.1016/j.foodqual.2020.103982

92. Deroy O, Reade B, Spence C. The insectivore's dilemma, and how to take the West out of it. *Food Qual Prefer.* (2015) 44:44–55. doi: 10.1016/j.foodqual.2015.02.007

93. Dossey AT, Tatum JT, McGill WL. Modern insect-based food industry: Current status, insect processing technology, and recommendations moving forward. In: *Insects as sustainable food ingredients: Production, processing, and food applications*. Dossey AT, Morales-Ramos JA, Guadalupe Rojas M, Eds. London, UK: Academic Press. (2016) p. 113–152. doi: 10.1016/B978-0-12-802856-8.00005-3

94. Parniakov O, Mikhrovska M, Wiktor A, Alles M, Ristic D, Bogusz R, et al. Insect processing for food and feed: a review of drying methods. *Dry Technol.* (2021). doi: 10.1080/07373937.2021.1962905

95. Bußler S, Rumpold BA, Fröhling A, Jander E, Rawel HM, Schlüter OK, et al. Cold atmospheric pressure plasma processing of insect flour from Tenebrio molitor: impact on microbial load and quality attributes in comparison to dry heat treatment. *Innov Food Sci Emerg Technol.* (2016) 36:277–86. doi: 10.1016/j.ifset.2016.07.002

96. Melgar-lalanne G. Edible insects processing: traditional and innovative technologies. *Compr Rev Food Sci Food Saf.* 18:1166–91. doi: 10.1111/1541-4337.12463

97. Kamalapuram SK, Handral H, Choudhury D. Cultured meat prospects for a billion! Foods. (2021) 10:2922. doi: 10.3390/foods10122922

98. Reiss J, Robertson S, Suzuki M. Cell sources for cultivated meat: applications and considerations throughout the production workflow. *Int J Mol Sci.* (2021) 22:7513. doi: 10.3390/ijms22147513

99. Balasubramanian B, Liu W, Pushparaj K, Park S. The epic of in vitro meat production—a fiction into reality. *Foods.* (1395) (2021):10. doi: 10.3390/foods10061395

100. Bodiou V, Moutsatsou P, Post MJ. Microcarriers for upscaling cultured meat production. *Front Nutr.* (2020) 7:10. doi: 10.3389/fnut.2020.00010

101. Seah JSH, Singh S, Tan LP, Choudhury D. Scaffolds for the manufacture of cultured meat. *Crit Rev Biotechnol.* (2022) 42:311–23. doi: 10.1080/07388551.2021.1931803

102. Bryant CJ. Culture, meat, and cultured meat. *J Anim Sci.* (2020) 98:1–7. doi: 10.1093/jas/skaa172

103. Fraeye I, Kratka M, Vandenburgh H, Thorrez L. Sensorial and nutritional aspects of cultured meat in comparison to traditional meat: Much to be inferred. *Front Nutr.* (2020) 7:35. doi: 10.3389/fnut.2020.00035

104. Rosenfeld DL, Tomiyama AJ. Would you eat a burger made in a petri dish? Why people feel disgusted by cultured meat. *J Environ Psychol.* (2022) 80:101758. doi: 10.1016/j.jenvp.2022.101758

105. Guan X, Lei Q, Yan Q, Li X, Zhou J, Du G, et al. Trends and ideas in technology, regulation and public acceptance of cultured meat. *Futur Foods.* (2021) 3:100032. doi: 10.1016/j.fufo.2021.100032

106. Zhang G, Chen J, Zhao X, Li X, Du G, Zhou J, et al. Challenges and possibilities for bio-manufacturing cultured meat. *Trends Food Sci Technol.* (2020) 97:443–50. doi: 10.1016/j.tifs.2020.01.026

107. Ramachandraiah K. Potential development of sustainable 3D-printed meat analogues: a review. *Sustain.* (2021) 13:938. doi: 10.3390/su13020938

108. Baiano A. 3D printed foods: A comprehensive review on technologies, nutritional value, safety, consumer attitude, regulatory framework, and economic and sustainability issues .*Food Rev Int.* (2020) 38:986–1016. doi: 10.1080/87559129.2020.1762091

109. Mikula K, Skrzypczak D, Izydorczyk G, Warchoł J, Moustakas K, Chojnacka K, et al. 3D printing filament as a second life of waste plastics—a review. *Environ Sci Pollut Res.* (2020) 12321–33. doi: 10.1007/s11356-020-10657-8

110. Tomašević I, Putnik P, Valjak F, Pavlić B, Šojić B, Bebek Markovinović A, et al. 3D printing as novel tool for fruit-based functional food production. *Curr Opin Food Sci.* (2021) 41:138–45. doi: 10.1016/j.cofs.2021.03.015

111. Verma VK, Kamble SS, Ganapathy L, Belhadi A, Gupta S. 3D Printing for sustainable food supply chains: Modelling the implementation barriers. *Int J Logist Res Appl.* (2022). doi: 10.1080/13675567.2022.2037125 (in press).

112. Zhang JY, Pandya JK, McClements DJ, Lu J, Kinchla AJ. Advancements in 3D food printing: A comprehensive overview of properties and opportunities. *Crit Rev Food Sci Nutr.* (2021). doi: 10.1080/10408398.2021.1878103

113. Pereira T, Barroso S, Gil MM. Food texture design by 3D printing: A review. *Foods.* (2021) 10:320. doi: 10.3390/foods10020320

114. Demei K, Zhang M, Phuhongsung P, Mujumdar AS. 3D food printing: Controlling characteristics and improving technological effect during food processing. *Food Res Int.* (2022) 156:111120. doi: 10.1016/j.foodres.2022.111120

115. Mantihal S, Kobun R, Lee BB. 3D food printing of as the new way of preparing food: a review. *Int J Gastron Food Sci.* (2020) 22:100260. doi: 10.1016/j.ijgfs.2020.100260

116. Mantihal S, Prakash S, Bhandari B. Texture-modified 3D printed dark chocolate: sensory evaluation and consumer perception study. *J Texture Stud.* (2019) 50:386–99. doi: 10.1111/jtxs.12472

117. Baumer KM, Lopez JJ, Naidu SV, Rajendran S, Iglesias MA, Carleton KM, et al. Visualizing 3D imagery by mouth using candy-like models. *Sci Adv.* (2021) 7:691–719. doi: 10.1126/sciadv.abh0691

118. Jiang H, Yu X, Fang R, Xiao Z, Jin Y. 3D printed mold-based capsaicin candy for the treatment of oral ulcer. *Int J Pharm.* (2019) 568:118517. doi: 10.1016/j.ijpharm.2019.118517

119. Motoki K, Park J, Spence C, Velasco C. Contextual acceptance of novel and unfamiliar foods: insects, cultured meat, plant-based meat alternatives, and 3D printed foods. *Food Qual Prefer.* (2022) 96:104368. doi: 10.1016/j.foodqual.2021.104368

120. Manstan T, McSweeney MB. Consumers' attitudes towards and acceptance of 3D printed foods in comparison with conventional food products. *Int J Food Sci Technol.* (2020) 55:323–31. doi: 10.1111/ijfs.14292

121. Wang T, Kaur L, Furuhata Y, Aoyama H, Singh J. 3D printing of textured soft hybrid meat analogues. *Foods.* (2022) 11:478. doi: 10.3390/foods11030478

122. Dick A, Bhandari B, Prakash S. 3D printing of meat. *Meat Sci.* (2019) 153:35–44. doi: 10.1016/j.meatsci.2019.03.005

123. Portanguen S, Tournayre P, Sicard J, Astruc T, Mirade PS. Toward the design of functional foods and biobased products by 3D printing: a review. *Trends Food Sci Technol.* (2019) 86:188–98. doi: 10.1016/j.tifs.2019.02.023

124. Ghazal AF, Zhang M, Bhandari B, Chen H. Investigation on spontaneous 4D changes in color and flavor of healthy 3D printed food materials over time in response to external or internal pH stimulus. *Food Res Int.* (2021) 142:110215. doi: 10.1016/j.foodres.2021.110215

125. Otero P, Garcia-Oliveira P, Carpena M, Barral-Martinez M, Chamorro F, Echave J, et al. Applications of by-products from the olive oil processing: Revalorization strategies based on target molecules and green extraction technologies. *Trends Food Sci Technol.* (2021) 116:1084–104. doi: 10.1016/j.tifs.2021.09.007

126. Berbel J, Posadillo A. Review and analysis of alternatives for the valorisation of agro-industrial olive oil by-products. *Sustain.* (2018) 10:237. doi: 10.3390/su10010237

127. Caldeira C, Laurentiis D, Corrado V, van Holsteijn S, Sala F, Quantification S, et al. (2019). Of food waste per product group along the food supply chain in the European Union: a mass flow analysis. *Resour Conserv Recycl.* 149:479–88. doi: 10.1016/j.resconrec.2019.06.011

128. Garcia-Garcia G, Stone J, Rahimifard S. Opportunities for waste valorisation in the food industry – a case study with four UK food manufacturers. *J Clean Prod.* (2019) 211:1339–56. doi: 10.1016/j.jclepro.2018.11.269

129. Ahmad Magry M, Narula SA. Sustainability of agri-food supply chains through innovative waste management models. In: *Valorization of Agri-Food Wastes and By-Products*. Elsevier. (2021) p. 591–605. doi: 10.1016/B978-0-12-824044-1.00039-8

130. Comunian TA, Silva MP, Souza CJF. The use of food by-products as a novel for functional foods: their use as ingredients and for the encapsulation process. *Trends Food Sci Technol.* (2021) 108:269–80. doi: 10.1016/j.tifs.2021.01.003

131. Antonic B, Jancikova S, Dordevic D, Tremlova B. Apple pomace as food fortification ingredient: a systematic review and meta-analysis. *J Food Sci.* (2020) 85:2977–85. doi: 10.1111/1750-3841.15449

132. Coelho M, Pereira R, Rodrigues AS, Teixeira JA, Pintado ME. Extraction of tomato by-products' bioactive compounds using ohmic technology. *Food Bioprod Process.* (2019) 117:329–39. doi: 10.1016/j.fbp.2019.08.005

133. Mwaurah PW, Kumar S, Kumar N, Panghal A, Attkan AK, Singh VK, et al. Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: A review. *Compr Rev Food Sci Food Saf.* (2020) 19:2421–46. doi: 10.1111/1541-4337.12598

134. Öztürk T, Turhan S. Physicochemical properties of pumpkin (Cucurbita pepo L.) seed kernel flour and its utilization in beef meatballs as a fat replacer and functional ingredient. *J Food Process Preserv.* (2020) 44:1–9. doi: 10.1111/jfpp.14695

135. Baldissera C, Hoppe A, Carlini NRBS, Sant'Anna, V. Factors influencing consumers' attitudes towards the consumption of grape pomace powder. *Appl Food Res.* (2022) 2:100103. doi: 10.1016/j.afres.2022.100103

136. Raposo A, Coimbra A, Amaral L, Gonçalves A, Morais Z. Eating jellyfish: safety, chemical and sensory properties. *J Sci Food Agric.* (2018) 98:3973–81. doi: 10.1002/jsfa.8921

137. Youssef J, Keller S, Spence C. Making sustainable foods (such as jellyfish) delicious. *Int J Gastron Food Sci.* (2019) 16:100141. doi: 10.1016/j.ijgfs.2019.100141

138. Amaral L, Raposo A, Morais Z, Coimbra A. Jellyfish ingestion was safe for patients with crustaceans, cephalopods, and fish allergy. *Asia Pac Allergy.* (2018) 8. doi: 10.5415/apallergy.2018.8.e3

139. Hsieh Y-HP, Leong FM, Rudloe J. Jellyfish as food. In: *Jellyfish Blooms: Ecological and Societal Importance*. Purcell JE, Graham WM, Dumont HJ, Eds. Dordrecht: Springer. (2001) p. 11–17. doi: 10.1007/978-94-010-0722-1_2

140. Ranasinghe RASN, Wijesekara WLI, Perera PRD, Senanayake SA, Pathmalal MM, Marapana RAUJ, et al. Nutritional value and potential applications of jellyfish. *J Aquat Food Prod Technol.* (2022) 31:445–82. doi: 10.1080/10498850.2022.2060717

141. Blikra MJ, Altintzoglou T, Løvdal T, Rognså G, Skipnes D, Skåra T, et al. Seaweed products for the future: using current tools to develop a sustainable food industry. *Trends Food Sci Technol.* (2021) 118:765–76. doi: 10.1016/j.tifs.2021.11.002

142. Choudhary B, Chauhan OP, Mishra A. Edible seaweeds: a potential novel source of bioactive metabolites and nutraceuticals with human health benefits. *Front Mar Sci.* (1422) (2021):8. doi: 10.3389/fmars.2021.740054

143. Sidari R, Tofalo RA. Comprehensive overview on microalgal-fortified/based food and beverages. *Food Rev Int.* (2019) 35:778–805. doi: 10.1080/87559129.2019.1608557

144. Bernaerts TMM, Gheysen L, Foubert I, Hendrickx ME, Van Loey AM. The potential of microalgae and their biopolymers as structuring ingredients in food: A review. *Biotechnol Adv.* (2019) 37:107419. doi: 10.1016/j.biotechadv.2019.107419

145. Fabris M, Abbriano RM, Pernice M, Sutherland DL, Commault AS, Hall CC, et al. Emerging technologies in algal biotechnology: toward the establishment of a sustainable, algae-based bioeconomy. *Front Plant Sci.* (2020) 11:1–22. doi: 10.3389/fpls.2020.00279

146. Duarte IM, Marques SC, Leandro SM, Calado R. An overview of jellyfish aquaculture: for food, feed, pharma and fun. *Rev Aquac.* (2022) 14:265–87. doi: 10.1111/raq.12597

147. FAO. *Thinking About the Future of Food*. (2022).

148. Echave J, Otero P, Garcia-Oliveira P, Munekata PES, Pateiro M, Lorenzo JM, et al. Seaweed-derived proteins and peptides: promising marine bioactives. *Antioxidants.* (2022) 11:176. doi: 10.3390/antiox11010176

149. Defraeye T, Shrivastava C, Berry T, Verboven P, Onwude D, Schudel S, et al. Digital twins are coming: will we need them in supply chains of fresh horticultural produce? Trends Food Sci. *Technol.* (2021) 109:245–58. doi: 10.1016/j.tifs.2021.01.025

150. Jagtap S, Bhatt C, Thik J, Rahimifard S. Monitoring potato waste in food manufacturing using image processing and internet of things approach. *Sustainability.* (2019) 11:3173. doi: 10.3390/su11113173

151. Chauhan C, Dhir A, Akram MU, Salo J. Food loss and waste in food supply chains. A systematic literature review and framework development approach. *J Clean Prod.* (2021) 295:126438. doi: 10.1016/j.jclepro.2021.126438

152. Kler R, Elkady G, Rane K, Singh A, Hossain MS, Malhotra D, et al. Machine learning and artificial intelligence in the food industry: a sustainable approach. *J Food Qual.* (2022) (2022):1–9. doi: 10.1155/2022/8521236

153. Freitas LC, Barbosa JR, Costa d, Bezerra ALC, Pinto FWF, Carvalho Junior RHH, et al. Waste to sustainable industry: how can agro-industrial wastes help in the development of new products? *Resour Conserv Recycl.* (2021) 169:105466. doi: 10.1016/j.resconrec.2021.105466

154. Montagner GE, Ribeiro MF, Cadoná FC, Franco C, Gomes P. Liposomes loading grape seed extract: a nanotechnological solution to reduce wine-making waste and obtain health-promoting products. *Futur Foods.* (2022) 5:100144. doi: 10.1016/j.fufo.2022.100144

155. Jagadiswaran B, Alagarasan V, Palanivelu P, Theagarajan R, Moses JA, Anandharamakrishnan C, et al. Valorization of food industry waste and by-products using 3D printing: a study on the development of value-added functional cookies. *Futur Foods.* (2021) 4:100036. doi: 10.1016/j.fufo.2021.100036

156. Mao G, Wu D, Wei C, Tao W, Ye X, Linhardt RJ, et al. Reconsidering conventional and innovative methods for pectin extraction from fruit and vegetable waste: Targeting rhamnogalacturonan I. *Trends Food Sci Technol.* (2019) 94:65–78. doi: 10.1016/j.tifs.2019.11.001

157. Oliveira D, Giordani CF, Gurak D, Cladera-Olivera PD, Marczak F, Extraction LDF, et al. Of pectin from passion fruit peel using moderate electric field and conventional heating extraction methods. *Innov Food Sci Emerg Technol.* (2015) 29:201–8. doi: 10.1016/j.ifset.2015.02.005

158. Bruno SF, Ekorong FJAA, Karkal SS, Cathrine MSB, Kudre TG. Green and innovative techniques for recovery of valuable compounds from seafood by-products and discards: a review. *Trends Food Sci Technol.* (2019) 85:10–22. doi: 10.1016/j.tifs.2018.12.004

159. Iriti M, Varoni EM, Vitalini S. Healthy diets and modifiable risk factors for non-communicable diseases—the European perspective. *Foods.* (2020) 9:940. doi: 10.3390/foods9070940

160. Stewart-Knox BJ, Markovina J, Rankin A, Bunting BP, Kuznesof S, Fischer ARH, et al. Making personalised nutrition the easy choice: Creating policies to break down the barriers and reap the benefits. *Food Policy.* (2016) 63:134–44. doi: 10.1016/j.foodpol.2016.08.001

161. Cropotova J, Suciu G, Vulpe AA. Strategic approach towards changing consumer eating behavior through a novel e-platform "Chef2plate." In: *Advances in intelligent systems and computing.* Rocha Á, Correia A, Adeli H, Reis L, Costanzo S, Eds. Cham: Springer. (2017) 463–9. doi: 10.1007/978-3-319-56535-4_47

162. Kellogg RA, Dunn J, Snyder MP. Personal omics for precision health. *Circ Res.* (2018) 122:1169–71. doi: 10.1161/CIRCRESAHA.117.310909

163. Gupta PD. Pharmacogenetics, pharmacogenomics and ayurgenomics for personalized medicine: a paradigm shift. *Indian J Pharm Sci.* (2015) 77:135. doi: 10.4103/0250-474X.156543

164. Gan J, Siegel JB, German JB. Molecular annotation of food – towards personalized diet and precision health. *Trends Food Sci Technol.* (2019) 91:675–80. doi: 10.1016/j.tifs.2019.07.016

165. Prasher B, Aggarwal S, Mandal AK, Sethi TP, Deshmukh SR, Purohit SG, et al. Whole genome expression and biochemical correlates of extreme constitutional types defined in Ayurveda. *J Transl Med.* (2008) 6:1–12. doi: 10.1186/1479-5876-6-48

166. Dey S, Pahwa P. Prakriti and its associations with metabolism, chronic diseases, and genotypes: possibilities of new born screening and a lifetime of personalized prevention. *J Ayurveda Integr Med.* (2014) 5:15. doi: 10.4103/0975-9476.128848

167. Prasher B, Gibson G, Mukerji M. Genomic insights into ayurvedic and western approaches to personalized medicine. *J Genet (2016) 951 95.* (2016) 209–28. doi: 10.1007/s12041-015-0607-9

168. Shifa K, Shobhana MC, Ayurnutrigenomics LVR. – a step towards personalized nutrition. *Indian J Appl Res.* (2022) 12:8–10. doi: 10.36106/ijar

169. Banerjee S, Debnath P, Debnath PK. Ayurnutrigenomics: ayurveda-inspired personalized nutrition from inception to evidence. *J Tradit Complement Med.* (2015) 5:228–33. doi: 10.1016/j.jtcme.2014.12.009

170. Ordovas JM, Ferguson LR, Tai ES, Mathers JC. Personalised nutrition and health. *BMJ.* (2018) 361. doi: 10.1136/bmj.k2173

171. Stewart-Knox BJ, Bunting BP, Gilpin S, Parr HJ, Pinhão S, Strain JJ, et al. toward genetic testing and personalised nutrition in a representative sample of European consumers. *Br J Nutr.* (2008) 101:982–9. doi: 10.1017/S0007114508055657

172. Heide M, Olsen SO. Co-production and time use. Influence on product evaluation. *Appetite.* (2011) 56:135–42. doi: 10.1016/j.appet.2010.12.001

173. Kole APW, Altintzoglou T, Schelvis-Smit RAAM, Luten JB. The effects of different types of product information on the consumer product evaluation for fresh cod in real life settings. *Food Qual Prefer.* (2009) 20:187–94. doi: 10.1016/j.foodqual.2008.09.003

174. Tiozon RJN, Fernie AR, Sreenivasulu N. Meeting human dietary vitamin requirements in the staple rice via strategies of biofortification and post-harvest fortification. *Trends Food Sci Technol.* (2021) 109:65–82. doi: 10.1016/j.tifs.2021.01.023

175. Olson R, Gavin-Smith B, Ferraboschi C, Kraemer K. Food fortification: the advantages, disadvantages and lessons from sight and life programs. *Nutrients.* (1118) (2021):13. doi: 10.3390/nu13041118

176. Picciotti U, Massaro A, Galiano A, Garganese F. Cheese fortification: review and possible improvements. *Food Rev Int.* (2021) 00:1–27. doi: 10.1080/87559129.2021.1874411

177. Eş I, Gavahian M, Marti-Quijal FJ, Lorenzo JM, Mousavi Khaneghah A, Tsatsanis C, et al. The application of the CRISPR-Cas9 genome editing machinery in food and agricultural science: current status, future perspectives, and associated challenges. *Biotechnol Adv.* (2019) 37:410–21. doi: 10.1016/j.biotechadv.2019.02.006

178. Feng Z, Zhang B, Ding W, Liu X, Yang DL, Wei P, et al. Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res.* (2013) 23:1229–32. doi: 10.1038/cr.2013.114

179. Zhang A, Liu Y, Wang F, Li T, Chen Z, Kong D, et al. Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Mol Breed.* (2019) 39:1–10. doi: 10.1007/s11032-019-0954-y

180. Li X, Zhou W, Ren Y, Tian X, Lv T, Wang Z, et al. High-efficiency breeding of early-maturing rice cultivars via CRISPR/Cas9-mediated genome editing. *J Genet Genomics.* (2017) 44:175–8. doi: 10.1016/j.jgg.2017.02.001

181. Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, et al. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnol J.* (2017) 15:207–16. doi: 10.1111/pbi.12603

182. Whitworth KM, Lee K, Benne JA, Beaton BP, Spate LD, Murphy SL, et al. Use of the CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. *Biol Reprod.* (2014) 91:78–9. doi: 10.1095/biolreprod.114.121723

183. Whitworth KM, Rowland RRR, Ewen CL, Trible BR, Kerrigan MA, Cino-Ozuna AG, et al. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nat Biotechnol.* (2016) 20–2. doi: 10.1038/nbt.3434

184. Xie Z, Pang D, Yuan H, Jiao H, Lu C, Wang K, et al. Genetically modified pigs are protected from classical swine fever virus. *PLoS Pathog.* (2018) 14:e1007193. doi: 10.1371/journal.ppat.1007193

185. Whitworth KM, Rowland RRR, Petrovan V, Sheahan M, Cino-Ozuna AG, Fang Y, et al. Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Res.* (2019) 28:21–32. doi: 10.1007/s11248-018-0100-3

186. Farid M, Cao J, Lim Y, Arato T, Kodama K. Exploring factors affecting the acceptance of genetically edited food among youth in Japan. *Int J Environ Res Public Health.* (2935) (2020):17. doi: 10.3390/ijerph17082935

187. Jain, A., Kumari, N., and Jha, V. K. A. review on hydroponic system: hope and hype. In Recent advances in chemical sciences & biotechnology; Jha, A.K., Kumar, U., Eds, New Delhi Publishers, New Delhi, India, (2018), 143–148. ISBN 9385503634.

188. Martinez P, Ahmad R. AllFactory: An Aquaponics 4.0 transdisciplinary educational and applied research learning factory at the University of Alberta. In: *Proceedings of the Conference on Learning Factories.* (2021) p. 5–7. doi: 10.2139/ssrn.3857901

189. Huynh A, Chen YC. Agriculture 4.0: Building aquaponics system using solar power adapt to climate change in Vietnam. *Int Adv Res J Sci Eng Technol.* (2021) 8:27–31. doi: 10.17148/IARJSET.2021.8105

190. Kumar AR, Divya TM, Jayasudha LY, BSK, Sudha, PN Precision agriculture: A review on its techniques and technologies. *Int Res J Mod Eng Technol Sci.* (2020) 02:1326–32. Available online at: https://www.irjmets.com/uploadedfiles/paper/volume2/issue_9_september_2020/3926/1628083155.pdf

191. Spanaki K, Karafili E, Sivarajah U, Despoudi S, Irani Z. Artificial intelligence and food security: swarm intelligence of AgriTech drones for smart AgriFood operations. *Prod Plan Control.* (2021). doi: 10.1080/09537287.2021.1882688

192. Wolfert S, Ge L, Verdouw C, Bogaardt MJ. Big data in smart farming – a review. *Agric Syst.* (2017) 153:69–80. doi: 10.1016/j.agsy.2017.01.023

193. Mahroof K, Omar A, Rana NP, Sivarajah U, Weerakkody V. Drone as a Service (DaaS) in promoting cleaner agricultural production and Circular Economy for ethical Sustainable Supply Chain development. *J Clean Prod.* (2021) 287:125522. doi: 10.1016/j.jclepro.2020.125522

194. Gilmour DN, Nayga RM, Bazzani C, Price H. *Consumers' willingness to pay for hydroponic lettuce: A non-hypothetical choice experiment*. Fayetteville, NC, USA: University of Arkansas. (2018).

195. Miličić V, Thorarinsdottir R, Dos Santos M, Hančič MT. Commercial aquaponics approaching the European market: To consumers' perceptions of aquaponics products in Europe. *Water.* (2017) 9:80. doi: 10.3390/w9020080

196. Siegrist M, Hartmann C. Consumer acceptance of novel food technologies. *Nat Food.* (2020) 1:343–50. doi: 10.1038/s43016-020-0094-x

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Quinoa extruded snacks with probiotics: Physicochemical and sensory properties

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The consumption of probiotic foods has grown rapidly, and these are generally found in dairy matrices where their growth is favored. Therefore, this study aimed to develop a new probiotic snack made from quinoa and added with spore-forming probiotic bacteria in two concentrations of 0.3 and 0.35%. The probiotic was added by spraying, after the extrusion process, together with salt and oil, at 70°C under dry conditions. Bacterial viability, resistance to simulated gastric juice, physical, chemical, and sensory tests were then evaluated during 120 days of storage at room temperature (20°C) and compared to a controlled snack without probiotic. The probiotic *Bacillus coagulans* was tested for the molecular identification and inhibition of pathogenic bacteria. Viability assessment was remained above 10^7 CFU/g of snacks. The intestinal tract simulation resistance test showed a viability of 70%. The physicochemical and sensory properties evaluated had no significant changes during storage time compared to control snack. The results of the taxonomic analysis indicate that the analyzed strain has, on average, 98% identity in 98% of its length belonging to *Bacillus coagulans* and *Bacillus badius* species. The probiotic showed inhibition against pathogenic bacteria. The new snack with probiotic is stable during storage.

KEYWORDS

spore-forming bacteria, gastrointestinal tract, stability, *Bacillus coagulans*, non-dairy probiotics, quinoa snack

Introduction

Quinoa is considered a pseudograin rich in protein (13.8–16.5%), carbohydrates (52–69%), lipids (2–9.5%), fiber (7–9.7%), vitamins and minerals (3.4%), and has a good balance of essential amino acids (Abugoch, 2009). In addition to the macronutrient content, according to a study realized by Pereira et al. (2019), three varieties of quinoa (red, black, and white color), high contents of proteins, carbohydrates, fat-soluble compounds, the presence of tocopherols, and fatty acids were evidenced, being γ-tocopherol the main isoform and linoleic acid the one found in higher concentrations. These are bioactive compounds that can generate benefits to human health, especially preventing cancer, reducing the risk of cardiovascular, inflammatory, gastrointestinal diseases, and improving metabolic health (Navruz-Varli and Sanlier, 2016). Therefore, quinoa-based foods are of great interest to the food industry.

This grain can be used to produce an innovative probiotic food, as there is a growing interest in the consumption of non-dairy probiotic foods, due to milk shortages in some countries, vegetarian and vegan trends, problems with cholesterol, allergen content, and lactose intolerances (Konuray and Erginkaya, 2020). The snacks that exist in the market are mainly made from corn or rice starch, and these present low nutritional quality due to their high-calorie content and scarcity of nutrients (Saldanha do Carmo et al., 2019). Therefore, a new quinoa probiotic food can be a healthier snack option. In the category of functional foods, those containing probiotic microorganisms represent the largest segment in the market (de Almada et al., 2016). This is because several health benefits have been demonstrated such as: facilitating lactose digestion, protecting against gastrointestinal diseases, balancing the immune system, preventing and treating dermatological diseases, and protecting against colon cancer (Sucupira and Souza, 2019).

Probiotic microorganism strains must present and maintain characteristics that guarantee their growth and survival in the food that contains them or to which they are added, as well as during their transit through the stomach and small intestine, and their ability to adhere to the mucosa of the large intestine; among the main characteristics are: viability during food processing and storage, stability against gastric acids and bile, adherence to the intestinal mucosa, and production of antimicrobial substances (Cao et al., 2020).

This type of lactic acid bacteria is generally present in dairy foods, which presumes a challenge for their incorporation in dry matrices such as cookies, snacks, and pasta among others, because most probiotic microorganisms cannot survive after heat treatment such as *Lactobacillus* and *Bifidobacterium* and some *Saccharomyces* species (Tripathi and Giri, 2014), for this reason, spore-forming probiotics are a viable alternative.

Bacillus coagulans is a Gram-positive, facultative, anaerobic, non-pathogenic, spore-forming, and lactic acid-producing bacterium (Lee et al., 2019). It is heat resistant; the optimal growth temperature for *B. coagulans* is 35 to 57°C and the optimal growth pH is 4 to 7 (Šipailiene and Petraityte, 2018). Furthermore, Soares et al. (2019) compared the resistance to gastrointestinal fluids of three probiotic strains *Lactobacillus*, *Bifidobacterium*, and *Bacillus* that were included in solid and liquid foods, and found that the latter had survivals of over 80%. The probiotic strain *Bacillus coagulans* has a good antibacterial activity due to the production of bacteriocins; in this respect, Gu et al. (2015) found that *Bacillus coagulans* CGMCC 9951 showed good antibacterial activity against several indicator bacteria, namely, *Escherichia coli*, *Pasteurella multocida*, *Staphylococcus aureus*, *Streptococcus suis*, and *Listeria monocytogenes*. Although *B. coagulans* produces acid, it does not produce gas from maltose, raffinose, mannitol, and sucrose fermentation; in addition to lactic acid production, some strains also produce thermostable α-amylase. For this reason, *B. coagulans* is important from an industrial point of view (Konuray and Erginkaya, 2018).

Several studies report *Bacillus coagulans* counts in food between 10^6 and 10^9 CFU in food. *Bacillus coagulans* GBI-30, 6086 incorporated into a wheat flour-based functional pasta remained viable during the pasta making and cooking processes (∼10^9 CFU/100 g) (Marcial-Coba et al., 2019). Majeed et al. (2016a) in their study prepared a series of foods incorporated with *Bacillus coagulans*, obtaining counts above 10^7 CFU/g. In their research, Almada-Érix et al. (2022) proved that the probiotic *Bacillus coagulans* added to bread showed high resistance to the baking process and was above 10^7 CFU/g. Majzoobi et al. (2019) in the results of their study showed that in the production of symbiotic bread an acceptable number of GanedenBC30 (more than 10^6 CFU/g) was obtained even after storage for 3 days at room temperature.

Bacillus coagulans BC30 strain is commercial and proprietary, and it is recommended to investigate molecular identification because the commercial probiotic strain may undergo several changes during the continuous and multiple years of commercial production (Sanders et al., 2014), which may alter physiological characteristics due to the living nature of probiotics. For this reason, genetic and phenotypic changes, by accident or design, can affect the efficacy and/or safety of commercial probiotics.

This work seeks to determine the stability and quality of *Bacillus coagulans* reference BC30 incorporated in an expanded snack made from quinoa during processing and storage at room temperature in a modified atmosphere for 120 days. In addition, this research sought to evaluate the resistance of the snack with probiotics to simulated intestinal transit conditions. To validate the commercial strain of Bacillus coagulans, molecular identification and inhibition of pathogenic bacteria was performed.

Materials and methods

Materials

Bacterial cultures. The lyophilized strain of *Bacillus coagulans* GBI-30, 6086 was acquired through a commercial supplier (Ganeden BC30, Kerry USA). The quinoa was provided by Segalco S.A.S. (Popayán, Colombia). The microorganisms used—*Listeria monocytogenes* ATCC 49594, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 6538—were obtained from the von Humboldt gene bank of the Microbiology and Applied Biotechnology Research Group (MIBIA) of the Universidad del Valle (Cali, Colombia).

Development of a snack with probiotic addition from quinoa in the SEGALCO company

The snacks were provided by Seguridad Alimentaria de Occidente SEGALCO S.A.S., a company located in the city of Popayán, Colombia. All snacks were prepared following the company's methodology (probiotic concentration was selected by the company based on sensory analysis and production cost studies). The probiotic snacks were added by spray with two concentrations of *B. coagulans* spores at 0.3% (w/w) snack with probiotic 1 (SP1) and 0.35% snack with probiotic 2 (SP2), together with salt and oil, after the extrusion process. A control snack (SC) was used without the addition of probiotics. This concentration was based on the manufacturer's recommendations, preliminary studies, and other research (Adibpour et al., 2019). The samples were packed in a modified atmosphere packaging containing nitrogen, and the packaging was multilayer laminated, and water vapor permeability WVTR and oxygen transmission rate OTR of the packaging were 0.7 g/m^2.day and 1.0 cc/m^2.day, respectively, and stored at room temperature (20°C) for 120 days. A snack without SC probiotic was used as a control, during which time physical and chemical tests were measured. Furthermore, the viability of the probiotic was verified.

Molecular identification of *Bacillus coagulans* strain and antimicrobial activity

Molecular identification was performed to corroborate the strain as a probiotic belonging to the B*acillus* species and its antimicrobial activity against Gram-positive and Gram-negative bacteria.

Molecular identification of the probiotic *B. coagulans*

Total DNA was extracted using lysozyme (10 mg mL^{-1}) followed by the addition of the DNAzol reagent (Thermo Fisher Scientific). PCR was performed using a Gene Cycler (Bio-Rad). Purification of PCR fragments and sequencing by Sanger method, using primers 337F, 518F, 800R, and 1100R of the 16S ribosomal gene. Taxonomic analysis of the problem sequence used the BLAST tool (Basic Local Alignment Search Tool), from NCBI (National Center for Biotechnology Information), compared against the reference RNA database "refseq_rna." Taxonomic analysis of the problem sequence using the "Classifier" and "SeqMatch" tools hosted on the RDP (Ribosomal Data Project) website. The first tool is used to determine the taxonomy of the problem sequence; the second tool is used to identify the most similar sequences, in the RDP database, to the problem sequence. Both perform the comparison using RDP's own "16S rRNA training set 18" database.

Multiple alignments, using the multiple sequence comparison by log-expectation (MUSCLE) algorithm, of the problem sequence with the 30 most similar sequences were reported by BLAST. Generation of a phylogenetic tree was performed using the Tamura-Nei genetic distance model (TN93), with the "Neighbor-Joining" method and the "Bootstrap" method with 1000 replicates. The taxonomic analysis and classification of the sequence was based on that presented by Majeed et al., 2016b with some modifications.

Antimicrobial activity of the probiotic *B. coagulans* against pathogen bacteria

Antimicrobial activity was performed according to the methodology by Sen et al. (2010) with slight modifications. *Bacillus coagulans* commercial strain was seeded in Man, Rogosa and Sharpe (MRS) broth (Scharlau, Spain) for 12 hours, cells were obtained by centrifugation of the culture broth at 3.800 rpm for 15 min and seeded by depth on glucose yeast extract (GYE) agar then the agar was allowed to dry for 15 min, then discs of approximately 0.6 mm in diameter were obtained and placed in a 15 mL layer of Mueller Hinton (Merck, Germany), containing approximately 10^6 CFU/mL of the indicator strains previously grown in Tryptone Soy broth (TSA) (Scharlau, Spain) either *Salmonella typhimurium* ATCC 14028 or *Listeria monocytogenes* ATCC 49594 or *Bacillus cereus* ATCC 14579 or *Bacillus subtilis* ATCC 6633 or *Escherichia coli* ATCC 25922 or *Staphylococcus aureus* ATCC 6538. The plates were allowed to stand for 30 min to allow the sample to diffuse into the agar and were subsequently taken to incubation at 37°C for 24 h. After incubation, the zone of inhibition was measured and recorded in mm.

Characterization of physicochemical properties as a function of time

Water activity

Water activity was determined in triplicate at 20°C, using a dew point water activity meter (AquaLab Series 3TE, Decagon Devices, Inc. USA).

pH variation

pH values of SP1, SP2, and SC were determined in triplicate, each replicate in quintuplicate according to the AOAC Method No. 943.02, using an Orion pH meter, model Three Stars (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a model 2A04 penetration electrode (Analizador, São Paulo, Brazil).

Moisture

Moisture analysis of the snacks was performed by loss on drying (130°C/3 h), based on (AOAC Official Method, 930.15, 2005).

Colorimetric properties

The color of the extrudate was determined using Konica Minolta Spectrophotometer CM-5, controlled by SpectraMagic NX software, with D65 illuminant and 10° observer angle. The samples were conditioned with a food processor. Samples were analyzed in triplicate, taking ~5 g of sample. Luminosity (L) and chromaticity parameters a* (−a * = green and + a * = redness) and b * (−b * = blue and + b * = yellow) and color variation (ΔE) were measured according to the Equation (1).

$$\Delta E = [(L\ estandar - L\ muestra)^2 + (a\ estandar - a\ muestra)^2 + (b\ estandar - b\ muestra)^2]^{1/2} \quad (1)$$

Texture

Texture properties were measured as described by Li et al. (2019) with some modifications using a texture analyzer (Shimadzu EZ-L, USA) with a 5 kN load cell and a 5 mm flat-head cylindrical probe. The SP1, SP2, and SC extrudates were randomly divided into three groups. Five measurements were performed for each group and the results were reported as the average of these three groups for each extrusion series. Three texture quality parameters, hardness, crispness, and crunchiness, were obtained from the texture analysis test curve with time (s) on the x-axis and force (N) on the y-axis. Hardness (N) is defined as the maximum force, crispness is the total number of positive peaks, and crunchiness (N s) is the linear distance from the test curve.

Viability of the probiotic *Bacillus coagulans* during storage

Viable count of *B. coagulans* was performed according to the methodology of the probiotic supplier and Majeed et al. (2016a), with some modifications. About 1 g of SP1 or SP2 was dissolved in 199 ml of sterile saline (0.9% NaCl, w/v). Then 30 ml was taken in a sterile tube and brought for 30 min at 70°C in a water bath, then, cooled to approximately 45°C before pipetting, cultured on GYE agar at 37°C for 48 h, under aerobic conditions. Survival was determined in duplicate under two concentrations 0.3 and 0.35: w/w, at days 0, 10, 20, 40, 60, and 120.

Gastric and pancreatic juices

The evaluation of the survival of the probiotic *Bacillus coagulans* included in snack SP1 subjected to simulated gastric and enteric conditions was performed according to Bedani et al. (2013) with some modifications.

One gram of SP1 was dissolved in 199 mL of sterile phosphate buffered saline (PBS) pH 7.4, 10 mL of this solution was taken in triplicate into flasks, then adjusted to pH 2 with 1 N HCl, and pepsin (from porcine stomach mucosa, Sigma-Aldrich) was added to the samples to a concentration of 3 g/L. The flasks were incubated at 37°C, with shaking at 150 rpm (Thermo Scientific MAxQ-4450 Shaker, USA) for 3 h, giving rise to the simulated gastric phase. In this phase, 1 mL was taken and seeding was performed on GYE agar at 37°C for 48 h under aerobic conditions. Subsequently, the pH of the samples was increased to 6.8 using an alkaline 1N NaOH solution and 30 mL of sterile phosphate buffer solution (150 ml of 1 N NaOH 14 g of PO4H2Na.2 H2O and distilled water up to 1 L), then Bile (bovine bile, Sigma-Aldrich) and pancreatin (porcine pancreas, Sigma-Aldrich) were added until reaching a concentration of 0.6 g/L and 1 g/L, respectively, in this phase, the three samples were left for 3 hours. About 1 ml of sample was taken for seeding, similar to the previous phase. Counts are expressed in log CFU/g of SP1. Survival rate at t0 and tf is expressed according to Equation (2) (Guo et al., 2009).

$$\%\ survivability\ \frac{N log\ final\ (tiempo\ 6h)}{N \log\log\ final\ (tiempo\ 0h)} * \quad (2)$$

Where, *N* is the number of CFU (colony forming units).

Sensory

The sensory evaluation was carried out using the hedonic test methodology with 14 panelists from the SEGALCO S.A.S. factory, composed of five women and nine men between 25 and 35 years of age. The panelists evaluated the perception parameters of general appearance, color, texture, aroma, flavor, chewiness, and acidity of the samples, giving scores from 5 to 1 (5 points, extremely good and 1 point, extremely low). For the acidity parameter, 1 is not perceived and 5 is perceived as high acidity. The study was reviewed and approved by the ethics committee of the University of Cauca and informed consent was obtained from each subject before they participated in the study.

Statistical analyses

Statistical analyses were performed using Minitab V 18 statistical software. Viability assessment during storage was performed in duplicate and *in vitro* gastric simulation experiments were performed in triplicate. Results were expressed as means ± standard deviation. One-way ANOVA was used to compare the viability of *B. coagulans*, after 0, 10, 20, 40, 40, 60, and 120 days of storage at 20°C. For sensory analysis, a Kruskal–Wallis non-parametric test was performed. Significance was set at $p < 0.05$. Tukey was used to compare the concentrations with a snack control without probiotics. The graphs were made in the Oring program.

Results and discussion

Molecular identification and antimicrobial activity

Molecular identification

Multifocus sequence typing was performed on the *B. coagulans* BC30 samples. The RDP classifier determined that this is a sequence of a microorganism belonging to the genus *Bacillus*.

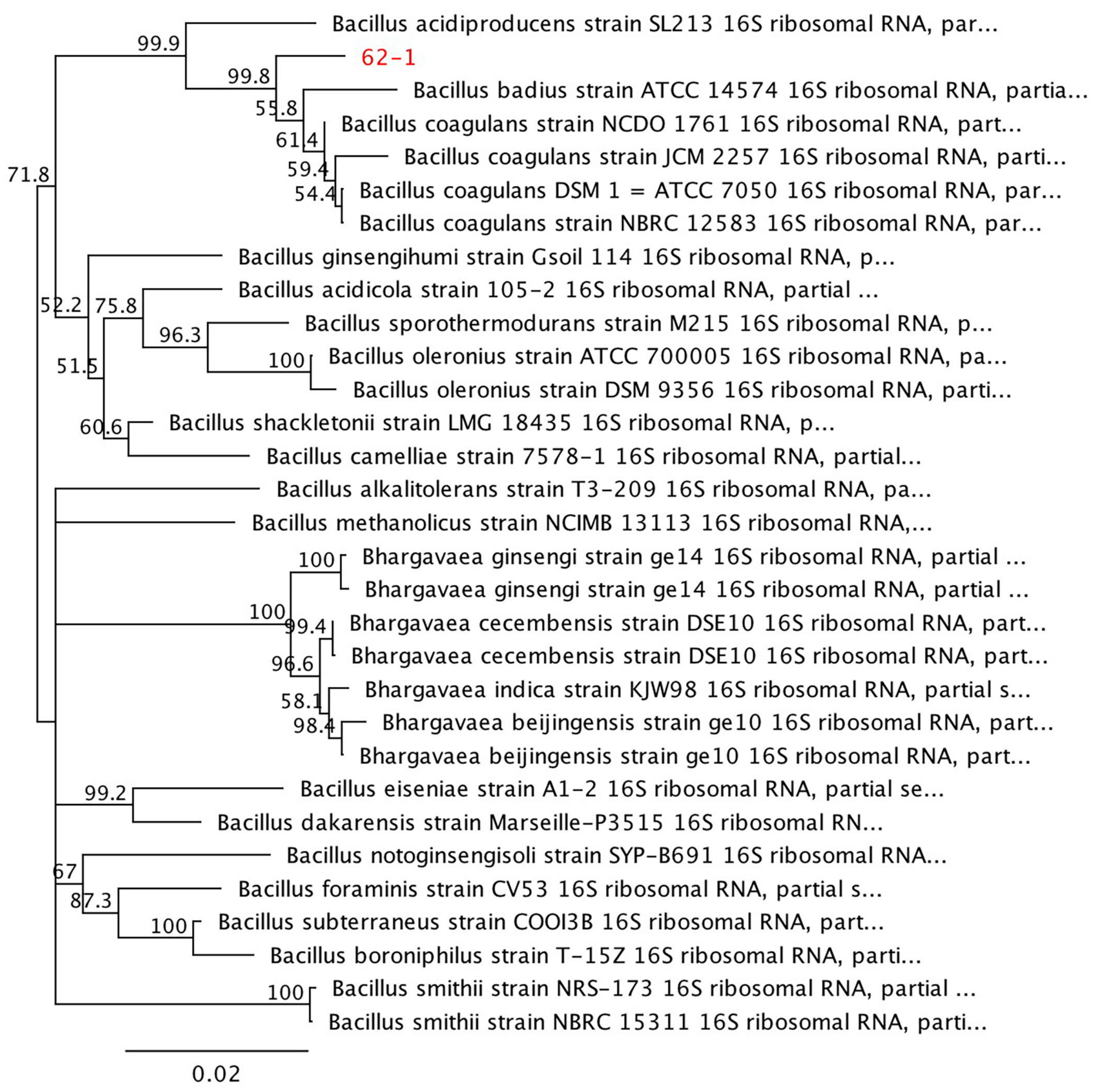

FIGURE 1
Phylogenetic tree by distances constructed from the thirty closest available sequences of culturable microorganisms according to the NCBI RefSeq_RNA database.

However, the resolving power of this classifier does not allow assigning a genus or species (S1-A). Comparison with the RDP 16S sequence database using the SeqMatch tool (S1-B) against the cultured isolate indicates that the assembled problem sequence (Scheme 1-S1-C) shows higher homology with sequences of *Bacillus coagulans* species. The results of the taxonomic analysis of the assembled 1525 bp problem sequence against the NCBI ref_seq database (S1-D) indicate that it has, on average, 98% identity over 98% of its length with 16S ribosomal gene sequences belonging to *Bacillus coagulans* and Bacillus badi species. The distance tree (Figure 1) constructed from the 30 closest available sequences of culturable microorganisms in the NCBI RefSeq_RNA database shows the analyzed sequence clusters with sequences of the species *Bacillus coagulans* (S1-E). In the case of this isolate, according to the results of searches in the different databases and the origin of the sample, it is concluded that it belongs to the species *Bacillus coagulans* (S1-E).

TABLE 1 Antimicrobial activity of *Bacillus coagulans* against test organisms.

Pathogen	R (mm)	
Listeria monocytogenes	4.35 ± 0.13	+ + +
Salmonella typhimurium	5.55 ± 0.27	+ + +
Bacillus cereus	4.1 ± 0.15	+ + +
Bacillus subtilis	3.9 ± 0.07	++
Escherichia coli	4.2 ± 0.05	+ + +
Staphylococcus aureus	3.8 ± 0.09	++

+, 1.0–2.0 mm; ++, 2.1–4.0 mm; + + +, más de 4 mm (Sen et al., 2010).

Pathogen growth inhibition

Probiotics of the genus *Bacillus* produce bioactive microbial secondary metabolites (bacteriocins), which regulate growth processes, applications, and/or exhibit response (regulation, inhibition, stimulation), in addition to bacteriocins, *B. coagulans* can also secrete other antimicrobial substances such as lactic acid (an important substance in the human intestine) and acetic acid (Cao et al., 2020).

As shown in Table 1, *Bacillus coagulans* generates an inhibitory halo against the growth of the pathogens under study. According to Bernardeau et al. (2017), the metabolites produced by the *Bacillus* genus are bacteriocins and inhibitory substances (e.g., subtilin and coagulin), an anionic antibacterial substance produced by *B. coagulans* that is a broad-spectrum agent against Gram-positive bacteria, which are involved in the transmission of foodborne diseases.

Furthermore, *Bacillus* can generate metabolites as antibiotics (e.g., surfactin, iturin, and fengycin) which may have an antibacterial or antifungal effect (Mondol et al., 2013). In their study, Abdhul et al. (2015) found that *Bacillus coagulans* strain

TABLE 2 Evaluation of the physicochemical characteristics of snacks with and without probiotic addition.

Sample	AT	pH	a_W	Moisture (%)	L	a*	b*	ΔE
SP1	0	$6.01^{a} \pm 0.03$	$0.20^{b} \pm 0.00$	$4.76^{c} \pm 0.01$	$61.95^{de} \pm 0.51$	$10.33^{bcde} \pm 0.02$	$37.37^{fg} \pm 0.28$	1.52
	10	$6.02^{a} \pm 0.03$	$0.19^{b} \pm 0.00$	$4.82^{c} \pm 0.01$	$62.71^{a} \pm 0.37$	$8.22^{g} \pm 0.11$	$39.00^{bc} \pm 0.61$	2.44
	20	$6.06^{a} \pm 0.07$	$0.21^{b} \pm 0.01$	$4.83^{bc} \pm 0.01$	$62.12^{bcde} \pm 0.03$	$9.17^{f} \pm 0.04$	$37.68^{ef} \pm 0.13$	1.86
	40	$6.01^{a} \pm 0.10$	$0.19^{b} \pm 0.01$	$4.86^{ab} \pm 0.01$	$62.63^{a} \pm 0.02$	$9.30^{f} \pm 0.02$	$37.54^{f} \pm 0.10$	1.73
	60	$6.01^{a} \pm 0.11$	$0.21^{ab} \pm 0.01$	$4.88^{bc} \pm 0.01$	$62.74^{a} \pm 0.05$	$10.24^{cde} \pm 0.01$	$35.72^{i} \pm 0.06$	2.96
	120	$5.52^{b} \pm 0.08$	$0.22^{a} \pm 0.02$	$4.90^{a} \pm 0.01$	$62.06^{cde} \pm 0.03$	$10.16^{de} \pm 0.04$	$35.14^{hi} \pm 0.04$	2.64
SP2	0	$5.99^{a} \pm 0.24$	$0.21^{b} \pm 0.00$	$4.82^{c} \pm 0.01$	$62.72^{a} \pm 0.05$	$10.57^{abcd} \pm 0.16$	$38.65^{cd} \pm 0.10$	0.06
	10	$6.02^{a} \pm 0.23$	$0.21^{b} \pm 0.01$	$4.81^{c} \pm 0.01$	$62.61^{a} \pm 0.03$	$10.86^{a} \pm 0.03$	$39.84^{a} \pm 0.05$	1.21
	20	$6.04^{a} \pm 0.23$	$0.19^{b} \pm 0.01$	$4.81^{bc} \pm 0.01$	$61.92^{e} \pm 0.01$	$10.28^{cde} \pm 0.06$	$37.60^{f} \pm 0.20$	1.37
	40	$6.04^{a} \pm 0.06$	$0.20^{b} \pm 0.02$	$4.89^{ab} \pm 0.01$	$62.44^{abc} \pm 0.02$	$9.05^{f} \pm 0.03$	$35.58^{gh} \pm 0.10$	2.63
	60	$6.02^{a} \pm 0.03$	$0.21^{ab} \pm 0.01$	$4.85^{bc} \pm 0.01$	$62.35^{abcde} \pm 0.01$	$10.02^{e} \pm 0.01$	$38.47^{cde} \pm 0.08$	0.74
	120	$5.55^{b} \pm 0.03$	$0.22^{a} \pm 0.00$	$4.92^{a} \pm 0.02$	$62.62^{a} \pm 0.08$	$10.54^{abcd} \pm 0.03$	$37.85^{def} \pm 0.03$	0.81
SC	0	$6.02^{a} \pm 0.09$	$0.19^{b} \pm 0.00$	$4.83^{c} \pm 0.02$	$62.72^{a} \pm 0.01$	$10.63^{abc} \pm 0.12$	$38.65^{cd} \pm 0.10$	-
	10	$5.99^{a} \pm 0.11$	$0.19^{b} \pm 0.00$	$4.82^{c} \pm 0.00$	$62.68^{a} \pm 0.13$	$10.83^{ab} \pm 0.08$	$39.74^{ab} \pm 0.14$	1.10
	20	$6.00^{a} \pm 0.07$	$0.19^{b} \pm 0.00$	$4.81^{bc} \pm 0.03$	$61.89^{e} \pm 0.07$	$10.32^{bcde} \pm 0.10$	$37.60^{f} \pm 0.20$	1.38
	40	$6.03^{a} \pm 0.21$	$0.19^{b} \pm 0.00$	$4.84^{ab} \pm 0.01$	$62.41^{abcd} \pm 0.01$	$10.07^{e} \pm 0.01$	$37.36^{fg} \pm 0.03$	1.44
	60	$6.02^{a} \pm 0.23$	$0.20^{ab} \pm 0.00$	$4.83^{bc} \pm 0.01$	$62.57^{ab} \pm 0.01$	$10.78^{ab} \pm 0.02$	$37.68^{ef} \pm 0.03$	0.99
	120	$5.52^{b} \pm 0.12$	$0.21^{a} \pm 0.00$	$4.89^{a} \pm 0.02$	$62.47^{abc} \pm 0.05$	$10.55^{abc} \pm 0.02$	$37.49^{f} \pm 0.03$	1.19

AT, analysis time (days); SP1, Snack probiotic concentration one; SP2, Snack probiotic concentration two; SC, Snack control. For each parameter, different letters indicate significant differences at $p < 0.05$.

FIGURE 2
Image of the snacks (SC, snack control; SP1, snack probiotic 1; SP2, snack probiotic 2).

[BDU3] generates antimicrobial inhibition on bacteria such as *Bacillus cereus* MTCC 430, *Staphylococcus aureus* MTCC 3160, and *Enterococcus sp*. MTCC 9728.

Moreover, as mentioned by Majeed et al. (2016b), commercial probiotic strains may have phenotypic changes during the continuous and multiple years of commercial production affecting their efficacy or safety; therefore, they evaluated the probiotic potential *in vitro*, finding that the *Bacillus coagulans* strain studied produces antimicrobial agents that inhibit the growth of pathogenic Gram-positive and Gram-negative bacteria.

pH variation of snacks with respect to storage time

As shown in Table 2, according to the analysis of variance, the two concentrations and storage time have no significant influence on the pH of snack samples. *B. coagulans* is a lactic acid-producing probiotic; however, during stress conditions such as low humidity, it is in the spore state, i.e., during the 120 days of storage, it has not initiated metabolic activity. According to Šipailiene and Petraityte (2018) the pH required by *Bacillus coagulans* strain is between 4 and 7, in snack samples SP1 and SP2 it was maintained between 6.01 and 5.52 during days 0 and 120, respectively. In order to survive and multiply, microorganisms have to maintain a stable pH in the cytoplasm, which ensures optimal functionality and integrity of the cytoplasm structure. In our study, the modified atmosphere packaging maintains the stable characteristics of the facultative anaerobic probiotic, since for this type of bacteria oxygen is a toxic element (Šipailiene and Petraityte, 2018).

Color

Color is one of the important characteristics of the acceptability of a food product. Table 2 shows the parameters of L* (Brightness), a* (Red/Green), and b* (blue/yellow). According to the analysis of variance, the color parameter L* is only affected by time and the parameters of a* and b* are influenced by concentration and by time ($p < 0.05$). The total color change (ΔE), which shows the color variation compared to the snack without probiotic addition and at day 0, was between 0.06 and 2.96, these values indicate no apparently perceptible changes during the storage time and the concentration of probiotic added. According to studies by Muñoz et al. (2022), in cereal mixtures extruded from quinoa, most of the population perceives a total color difference >3. Therefore, as shown in Figure 2, the addition of probiotics to the snack does not result in a significant color change. According to the results of Konuray and Erginkaya (2020), they had no change in color parameters over time in pasta added with probiotic lactic acid bacteria. Likewise, Akman et al. (2019) reported in their study that the enrichment of apple slices with *Lacticaseibacillus paracasei* had no significant effect on color changes in all dried apple samples.

Texture

Figure 3 shows the effect of the two concentrations and days of storage with respect to the control. According to the analysis of variance, the factors of concentration and time have no significant effect $p > 0.05$ on the strength (N), crunchiness, and crispness (NS) during the 120 days of storage, that is to say, that the SP1, SP2, and SC matrices do not have significant changes over time, due to the fact that the packaging maintains

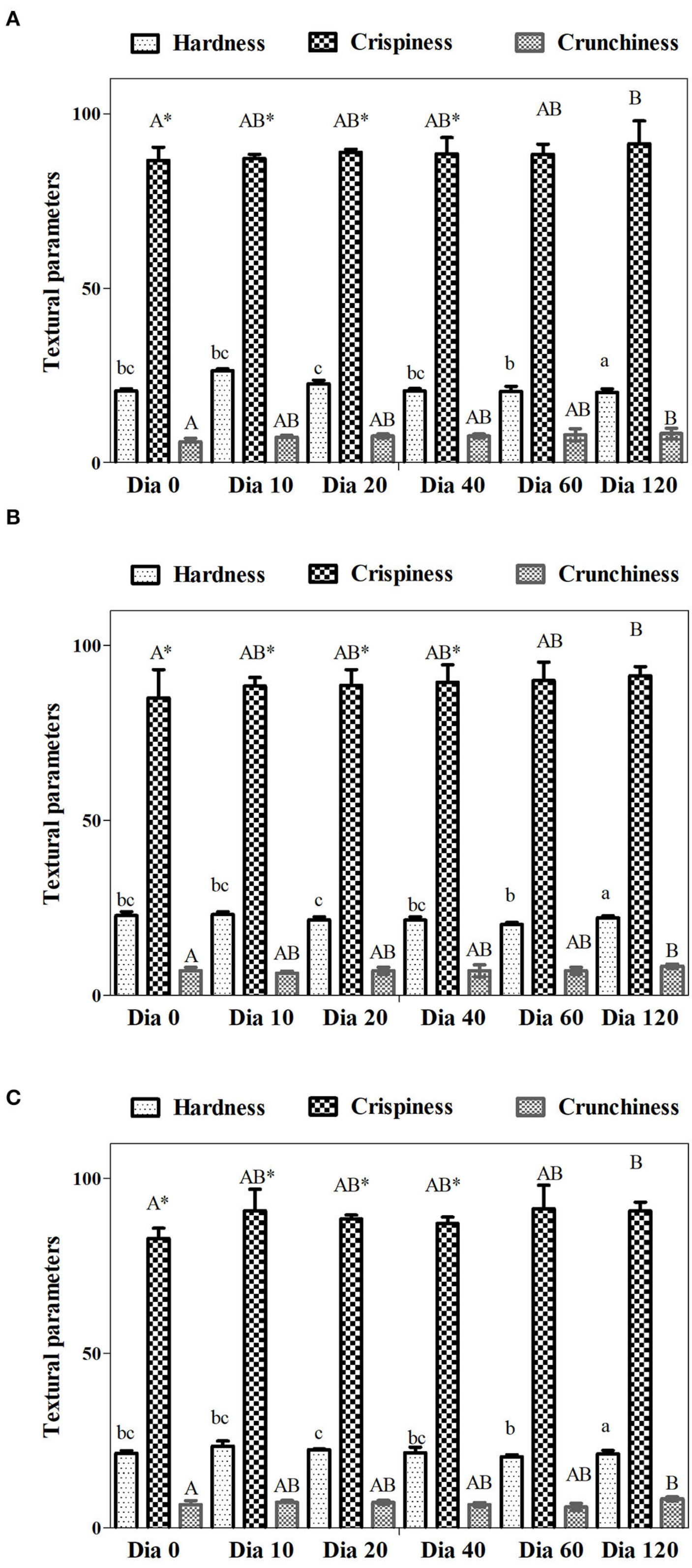

FIGURE 3
Textural properties (hardness, crunchiness, and crispiness) of quinoa flour extrudates obtained at different amounts of probiotics. ($n = 3$ for extrusion runs, $n = 3$ for texture measurements). **(A)** SP1; **(B)** SP2; **(C)** SC. Within hardness, crunchiness, and crispiness, values followed by different letters are significantly different ($p < 0.05$).

the humidity and water activity. According to Ananta et al. (2005), to maintain stability, water activity should not exceed 0.25 and moisture content should be 4–7%. In our study, aw and moisture contents were maintained between 0.19–0.22 and 4.76–4.92, respectively, during the 120 days of storage.

The extrusion process is a versatile and low-cost technology used for the production of cereal-based snacks, which transforms starch and protein-based solid materials into a viscoelastic fluid under high pressure and temperature conditions (Li et al., 2019). The addition of the probiotic does not significantly alter the texture parameters, which is corroborated in the sensory analysis, where no significant changes are perceived.

Viability of the *Bacillus coagulans* probiotic under storage conditions

The analysis is performed at room temperature, to prove that the probiotics incorporated in snacks made from quinoa remain under shelf-life conditions.

According to the analysis of variance, the two concentrations analyzed, do not present effect on the counts ($p > 0.05$), however, the different days analyzed do present effect on the count ($p < 0.05$). As shown in Table 3, the counts from 0 to 120 days are between 7.39 log CFU/g and 7.95 log CFU/g, respectively. During the period studied, the viability of the probiotic increased by 7.57% with respect to day 0, due to the fact that during the probiotic inclusion process no extreme drying or pressure processes are performed. It is possible that the sporulated probiotic strain does not affect its viability and remains during the period of analysis, without changing the organoleptic properties of the snack as corroborated in the sensory analysis, because the packaging maintains a water activity lower than 0.25, where the molecular mobility of the matrix is limited, an aspect that discourages metabolism; in this sense, according to studies by Ananta et al. (2005) in encapsulated bacteria, where the water activity was 0.2, bacterial viability after 45 days was higher than 10^5 CFU/g. During the 120 days, the probiotic remained stable, i.e., it did not grow exponentially, an aspect that allows preserving the physico-chemical characteristics in the snack, and similar results were obtained by Almada-Érix et al. (2022) in samples of bread added with *Bacillus coagulans* GBI-30 6086.

Furthermore, an increase in the count on days 60 and 120 is possibly due to some of the latent probiotics becoming viable and active. According to Zendeboodi et al. (2020), probiotics such as *Bacillus coagulans* are inactive probiotics. Although they are viable, they are not active and the rate of growth and/or generation of metabolites are almost stopped, they are called "pseudoprobiotics."

The *Bacillus coagulans* strain retained its viability during the 120 days of storage above 6 log CFU/g, which is the permitted limit for probiotic foods (Konuray and Erginkaya, 2020). *Bacillus coagulans* is a strain labeled as a GRAS food ingredient by the US FDA, it is a Gram-positive, microaerophilic, spore-producing *Bacillus coagulans*, which makes it heat resistant, and therefore a good choice for products with low humidity. According to studies conducted by Majeed et al. (2016a), the stability of *Bacillus coagulans* MTCC 5856 was analyzed in different food matrices after 12 months they obtained viability above 80%. Likewise, Majzoobi et al. (2019) obtained baked and then frozen food, added with Ganeden BC30 that retained a viability of 7.35 log CFU/g after 56 days of storage.

Gastric and pancreatic juices

Viability is the ability of these microorganisms to remain alive, both in the organism and in the consumer's intestine for a given time, in order to achieve the benefits of such foods.

The survival of freeze-dried *B. coagulans* in spore present in the SP1 subjected to simulated gastrointestinal conditions *in vitro* is shown in Table 4. Based on the results, the probiotic included in the snack resists the passage through the simulation. According to Casula and Cutting (2002), spores of the genus

TABLE 4 Tolerance of *Bacillus coagulans* to simulated gastric juice.

	Hours			
	0	**3**	**6**	**Survivability (%)**
SP1	6.74 ± 0.09	4.98 ± 0.09	4.72 ± 0.11	70.12 ± 1.08

Viable counts (log CFU/g) of SP1 at zero (0), three (3), and six (6) h.

TABLE 3 Evaluation of the viability of spore-forming bacteria in a quinoa snack during storage time at room temperature (20°C) and dry place.

Sample	**Storage time days**					
	0	10	20	40	60	120
SP1	$7.39^{d} \pm 0.04$	$7.43^{d} \pm 0.08$	$7.56^{bc} \pm 0.08$	$7.56^{cd} \pm 0.1$	$7.64^{b} \pm 0.05$	$7.95^{a} \pm 0.04$
SP2	$7.47^{d} \pm 0.01$	$7.41^{d} \pm 0.06$	$7.51^{bc} \pm 0.14$	$7.51^{cd} \pm 0.13$	$7.68^{b} \pm 0.09$	$7.93^{a} \pm 0.17$

Values are presented as mean ± SD ($n = 3$). For each parameter, different letters indicate significant differences at $p < 0.05$.

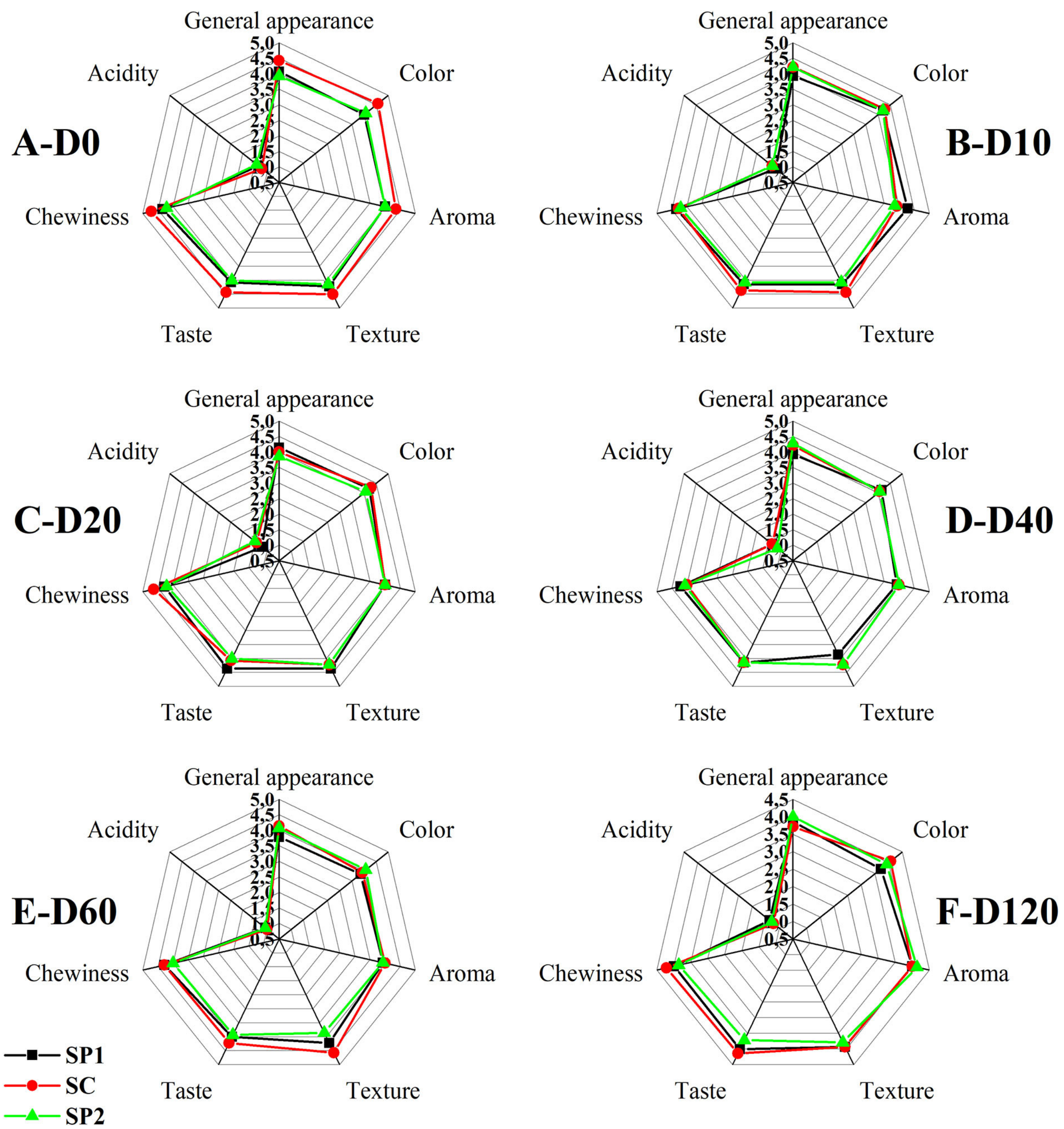

FIGURE 4
Sensory evaluation of samples (**A**, day 0; **B**, day 10; **C**, day 20; **D**, day 40; **E**, day 60; **F**, day 120; SP1, snack probiotic concentration one; SP2, probiotic concentration two, SC, control).

Bacillus can germinate and proliferate in the gut of mice, indicating that spores can germinate, grow and then sporulate in the gut. According to Cao et al. (2020), the life cycle of *Bacillus coagulans* in the intestinal tract is described as follows: initially, it enters as a spore and continues as such during the time it remains in the stomach for almost 3 h, later the spores germinate in the small intestine, and then the live bacteria will travel down to the large intestine and sporulate in the lower part.

In future studies, it is valuable to perform *in vivo* tests to demonstrate the resistance of snacks with probiotic addition.

According to the above, the population of the spore-forming probiotic showed a reduction from 0 to 3 h test of 1.76 and between the 3 to 6 h period the reduction was 0.26 log CFU/g. The greatest decrease in viability of this strain was observed in the gastric phase, suggesting its high sensitivity to simulated gastric juice containing HCl and pepsin. In studies performed with a similar strain, Sui et al. (2020) found higher survival values of the sporulated probiotic above 80%, and in our assay, the percentage is almost 70%, i.e., more than half passed the gastric phase. According to Marcial-Coba et al. (2019) in order for probiotics to provide a benefit, they must pass through the acidic conditions of the stomach, and the spores of *B. coagulans* and other *Bacillus* species remain in their spore form during passage through the stomach and duodenum then germinate in the jejunum and ileum, with subsequent transient colonization of the small intestine, where vegetative cells can produce active metabolites such as L (+) lactic acid and interact with the host and intestinal microbiota. Maathuis et al. (2010) investigated the survival of the GanedenBC30 spore during its passage through the upper gastrointestinal tract in a dynamic, validated, *in vitro* model of the stomach and small intestine. In their study, they also found that the survival of GanedenBC30 was 70%.

Sensory

A 5-point scale, 1 lowest and 5 highest, was used in this study. As shown in Figure 4, the panelists rated the SC, SP1, and SP2 between 4 and 5 for the quality parameters analyzed, with the exception of acidity, which for the three samples is 1, i.e., it is not perceived. There are no significant differences between the storage time and the control snack without probiotics. Our results corroborate that the physicochemical properties remain stable during the 120 days of analysis in metalized packaging in a modified atmosphere, so the panelists rated the snacks with high quality, both the control and those added with probiotics.

In studies conducted by Adibpour et al. (2019), they found no differences between the control and a product made with caramel and sporulated probiotic. Konuray and Erginkaya (2020) in their study with *Bacillus coagulans*-incorporated pasta found that sensory attributes were not affected during 6 months of storage.

Conclusion

The results of the study showed that the SP1 and SP2 quinoa probiotic snacks made with the addition of *B. coagulans* GBI-30 have a final concentration of approximately 10^7 UFC/g, and can be stored for 120 days at room temperature, low humidity, and aw: 0.2 ± 0.2 without affecting their quality. Sporulated *B. coagulans* bacteria showed inhibition against the pathogenic bacteria tested. Survival during simulated intestinal tract conditions in the food matrix SP1 showed 70%. The addition of the probiotic GBI-30 showed no statistically significant effect on the sensory characteristics, texture, color, acidity, and overall appearance of the samples during storage compared to the control snack. The probiotic snacks produced in our study can be considered an acceptable probiotic food after 120 days of storage, which can be realized at the industrial level. Due to the growing consumer interest in non-dairy probiotic food alternatives, the quinoa-based snack produced in this study is a viable alternative to a vegan probiotic food.

Data availability statement

The original contributions on the genetic identification test presented in the study are included in the article/Supplementary material, additional inquiries can be directed to the corresponding author(s).

Author contributions

JS and KM: methodology, formal analysis, investigation, and writing original draft. JH: methodology, formal analysis, and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.935425/full#supplementary-material

References

Abdhul, K., Ganesh, M., Shanmughapriya, S., Vanithamani, S., Kanagavel, M., Anbarasu, K., et al. (2015). Bacteriocinogenic potential of a probiotic strain *Bacillus coagulans* [BDU3] from Ngari. *Int. J. Biol. Macromol.* 79, 800–806. doi: 10.1016/j.ijbiomac.2015.06.005

Abugoch, J. L. E. (2009). "Quinoa (Chenopodium Quinoa Willd.): composition, chemistry, nutritional, and functional propertiesm," in *Advances in Food and Nutrition Research*, *1st Edn*, *Vol. 58*. Santiago de Chile: Elsevier Inc. doi: 10.1016/S1043-4526(09)58001-1

Adibpour, N., Hosseininezhad, M., and Pahlevanlo, A. (2019). Application of spore-forming probiotic Bacillus in the production of Nabat - a new functional sweetener. *LWT. Food Sci. Technol.* 113, 108277. doi: 10.1016/j.lwt.2019.108277

Akman, P. K., Uysal, E., Ozkaya, G. U., Tornuk, F., and Durak, M. Z. (2019). Development of probiotic carrier dried apples for consumption as snack food with the impregnation of Lactobacillus paracasei. *LWT. Food Sci. Technol.* 103, 60–68. doi: 10.1016/j.lwt.2018.12.070

Almada-Érix, C. N., Almada, C. N., Souza Pedrosa, G. T., Paulo Biachi, J., Bonatto, M. S., Schmiele, M., et al. (2022). Bread as probiotic carriers: resistance of *Bacillus coagulans* GBI-30 6086 spores through processing steps. *Food Res. Int.* 155, 111040. doi: 10.1016/j.foodres.2022.111040

Ananta, E., Volkert, M., and Knorr, D. (2005). Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. *Int. Dairy J.* 15, 399–409. doi: 10.1016/j.idairyj,.2004.08.004

AOAC Official Method, 930.15 (2005). *Official Methods of Analysis of AOAC INTERNATIONAL*, *18th Edn*. Gaithersburg, MD: AOAC INTERNATIONAL.

Bedani, R., Rossi, E. A., and Saad, S. M. I. (2013). Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under *in vitro* simulated gastrointestinal conditions. *Food Microbiol.* 34, 382–389. doi: 10.1016/j.fm.2013.01.012

Bernardeau, M., Lehtinen, M. J., Forssten, S. D., and Nurminen, P. (2017). Importance of the gastrointestinal life cycle of Bacillus for probiotic functionality. *J. Food Sci. Technol.* 54, 2570–2584. doi: 10.1007/s13197-017-2688-3

Cao, J., Yu, Z., Liu, W., Zhao, J., Zhang, H., and Zhai, Q. (2020). Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases. *J. Funct. Foods* 64, 103643. doi: 10.1016/j.jff.2019.103643

Casula, G., and Cutting, S. M. (2002). Bacillus probiotics: spore germination in the gastrointestinal tract. *Appl. Environ. Microbiol.* 68, 2344–2352. doi: 10.1128/AEM.68.5.2344-2352.2002

de Almada, C. N., Almada, C. N., Martinez, R. C. R., and Sant'Ana, A. S. (2016). Paraprobiotics: evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. *Trends Food Sci. Technol.* 58, 96–114. doi: 10.1016/j.tifs.2016.09.011

Gu, S., Bin, Z.hao, L. N., Wu, Y., Li, S. C., Sun, J. R., Huang, J. F., et al. (2015). Potential probiotic attributes of a new strain of *Bacillus coagulans* CGMCC 9951 isolated from healthy piglet feces. *World J. Microbiol. Biotechnol.* 31, 851–863. doi: 10.1007/s11274-015-1838-x

Guo, Z., Wang, J., Yan, L., Chen, W., Liu, X., ming, Zhang, H., et al. (2009). *In vitro* comparison of probiotic properties of *Lactobacillus* casei Zhang, a potential new probiotic, with selected probiotic strains. *LWT Food Sci. Technol.* 42, 1640–1646. doi: 10.1016/j.lwt.2009.05.025

Konuray, G., and Erginkaya, Z. (2018). Potential use of *Bacillus coagulans* in the food industry. *Foods.* 7, 92. doi: 10.3390/foods7060092

Konuray, G., and Erginkaya, Z. (2020). Quality evaluation of probiotic pasta produced with *Bacillus coagulans* GBI-30. *Innovative Food Sci. Emerg. Technol.* 66, 102489. doi: 10.1016/j.ifset.2020.102489

Lee, N. K., Kim, W. S., and Paik, H. D. (2019). Bacillus strains as human probiotics: characterization, safety, microbiome, and probiotic carrier. *Food Sci. Biotechnol.* 28, 1297–1305. doi: 10.1007/s10068-019-00691-9

Li, X., Masatcioglu, M. T., and Koksel, F. (2019). Physical and functional properties of wheat flour extrudates produced by nitrogen injection assisted extrusion cooking. *J. Cereal Sci.* 89, 102811. doi: 10.1016/j.jcs.2019.10 2811

Maathuis, A. J. H., Keller, D., and Farmer, S. (2010). Survival and metabolic activity of the GanedenBC30 strain of *Bacillus coagulans* in a dynamic *in vitro* model of the stomach and small intestine. *Beneficial Microbes.* 1, 31–36. doi: 10.3920/BM2009.0009

Majeed, M., Majeed, S., Nagabhushanam, K., Natarajan, S., and Ali, F. (2016a). Original article Evaluation of the stability of *Bacillus coagulans* MTCC 5856 during processing and storage of functional foods. *Int. J. Food Sci. Technol.* 32, 1–8. doi: 10.1111/ijfs.13044

Majeed, M., Nagabhushanam, K., Natarajan, S., Sivakumar, A., Eshuis-de Ruiter, T., Booij-Veurink, J., et al. (2016b). Evaluation of genetic and phenotypic consistency of *Bacillus coagulans* MTCC 5856: a commercial probiotic strain. *World J. Microbiol. Biotechnol.* 32, 1–12. doi: 10.1007/s11274-016-2027-2

Majzoobi, M., Aghdam, M. B. K., Eskandari, M. H., and Farahnaky, A. (2019). Quality and microbial properties of symbiotic bread produced by straight dough and frozen part-baking methods. *J. Texture Stud.* 50, 165–171. doi: 10.1111/jtxs.12386

Marcial-Coba, M. S., Pjaca, A. S., Andersen, C. J., Knøchel, S., and Nielsen, D. S. (2019). Dried date paste as carrier of the proposed probiotic *Bacillus coagulans* BC4 and viability assessment during storage and simulated gastric passage. *LWT Food Sci. Technol.* 99, 197–201. doi: 10.1016/j.lwt.2018.09.052

Mondol, M. A. M., Shin, H. J., and Islam, M. T. (2013). Diversity of secondary metabolites from marine bacillus species: chemistry and biological activity. *Marine Drugs* 11, 2846–2872. doi: 10.3390/md11082846

Muñoz, K. S., Parra, A. S., Roa, D. F., Hoyos, J. L., and Bravo, J. E. (2022). Physical and paste properties comparison of four snacks produced by high protein quinoa flour extrusion *Cooking* 6, 1–10. doi: 10.3389/fsufs.2022.852224

Navruz-Varli, S., and Sanlier, N. (2016). Nutritional and health benefits of quinoa (Chenopodium quinoa Willd.). *J. Cereal Sci.* 69, 371–376. doi: 10.1016/j.jcs.2016.05.004

Pereira, E., Encina-Zelada, C., Barros, L., Gonzales-Barron, U., Cadavez, V., and, C. F. R., et al. (2019). Chemical and nutritional characterization of *Chenopodium quinoa* Willd (quinoa) grains: a good alternative to nutritious food. *Food Chem.* 280, 110–114. doi: 10.1016/j.foodchem.2018.12.068

Saldanha do Carmo, C., Varela, P., Poudroux, C., Dessev, T., Myhrer, K., Rieder, A., et al. (2019). The impact of extrusion parameters on physicochemical, nutritional and sensorial properties of expanded snacks from pea and oat fractions. *LWT Food Sci. Technol.* 112, 108252. doi: 10.1016/j.lwt.2019.108252

Sanders, M. E., Klaenhammer, T. R., Ouwehand, A. C., Pot, B., Johansen, E., Heimbach, J. T., et al. (2014). Effects of genetic, processing, or product formulation changes on efficacy and safety of probiotics. *Ann. N. Y. Acad. Sci.* 1309, 1–18. doi: 10.1111/nyas.12363

Sen, R., Pal, D., Kodali, V. P., Das, S., and Ghosh, S. K. (2010). Molecular characterization and *in vitro* analyses of a sporogenous bacterium with potential probiotic properties. *Probiotics Antimicrob. Proteins* 2, 152–161. doi: 10.1007/s12602-010-9049-0

Šipailiene, A., and Petraityte, S. (2018). Encapsulation of probiotics: proper selection of the probiotic strain and the influence of encapsulation technology and materials on the viability of encapsulated microorganisms. *Probiotics Antimicrob. Proteins* 10, 1–10. doi: 10.1007/s12602-017-9347-x

Soares, M. B., Martinez, R. C. R., Pereira, E. P. R., Balthazar, C. F., Cruz, A. G., Ranadheera, C. S., et al. (2019). The resistance of Bacillus, Bifidobacterium, and Lactobacillus strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Res. Int.* 125, 108542. doi: 10.1016/j.foodres.2019.108542

Sucupira, M. I., and Souza, M. M. B. (2019). Prebiotics and probiotics - potential benefits in human nutrition and health. *Intech* 15, 1–15. doi: 10.5772/intechopen.89155

Sui, L., Zhu, X., Wu, D., Ma, T., Tuo, Y., Jiang, S., et al. (2020). *In vitro* assessment of probiotic and functional properties of *Bacillus coagulans* T242. *Food Biosci.* 36, 100675. doi: 10.1016/j.fbio.2020.100675

Tripathi, M. K., and Giri, S. K. (2014). Probiotic functional foods: survival of probiotics during processing and storage. *J. Funct. Foods* 9, 225–241. doi: 10.1016/j.jff.2014.04.030

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., and da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Curr. Opin. Food Sci.* 32, 103–123. doi: 10.1016/j.cofs.2020.03.009

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Camel milk protectiveness toward multiple liver disorders: A review

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Camel milk is known as the white gold of the desert because it contains within it a variety of nutrients which play a key role in the human diet. The health benefits of camel milk have been described for a variety of diseases such as diabetes, kidney disease, hepatitis, etc. including improved overall survival. A major health burden worldwide is liver diseases, and the ninth leading cause of death in Western countries is due to liver cirrhosis. Treatment is mostly ineffective for cirrhosis, fatty liver, and chronic hepatitis which are the most common diseases of the liver; furthermore current treatments carry the risk of side effects, and are often extremely expensive, particularly in the developing world. A systematic review of studies was performed to determine the association of consumption of camel milk on multiple diseases of the liver. The impact of camel milk on the laboratory tests related to the liver disorders, viral hepatitis, non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma (HCC) were evaluated. The consumption of camel milk was accompanied by modulation of the values of serum gamma-glutamyl transferase, aspartate aminotransferase, and alanine aminotransferase in persons who are at risk of liver disease. In the patients with chronic liver disease, it was observed that they have low rates of mortality and low chances of progression to cirrhosis when they consume camel milk. Therefore, in patients with liver diseases, the addition of camel milk to their normal daily diet plan should be encouraged. In this review, camel milk's impact on the different kinds of liver diseases or any disorder associated with liver functioning was evaluated. Camel milk has a therapeutic as well as a preventive role in the maintenance and improving the metabolic regulations of the body.

KEYWORDS
camel milk, liver disorders, hepatitis, non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma

Introduction

Milk is considered one of the most important foods for humans and animals, and acts as a complete diet due to its crucial components such as carbohydrates, proteins, fats, vitamins, and minerals (1–3). Milk composition is highly dependent, which can be greatly affected by many factors such as animal's health status, especially the mammary gland health, photoperiod effect of different seasons, animal's diet (for example a higher concentrate intake during dry season), genetic factors, and the temperature of milk storage (4–6). Among varieties of milk available, camel milk is also known as the "white gold" of the desert as it contains essential nutrients that play an important role. In desert areas worldwide, Dromedary camels (*Camelus dromedarius*) are habitant, and the milk is available in the form of many products, of which cheese, powdered camel milk, coffee, and ice cream are sold in many developed countries (7, 8). Its milk is also available commercially at low prices. The production of camel milk has increased 4.6 times globally and the production level has reached 2.9 million tons between 1961 and 2017 (9). Camel milk has a great nutritive value, as well as the value of its functional ingredients in it (10). Table 1 highlights the nutritional components of camel milk. Like other kinds of milk, it also consists of all essential nutrients. The composition of cow milk is compatible with that of camel milk. It contains more zinc, iron, vitamin C, and E as compared to other kinds of milk (11). It has an essential role in the improvement of the immune system. It can fight against major diseases and this attribute is because it contains major proteins such as lactoferrin, peptidoglycan, antibodies immunoglobulins and some enzymes such as lysozymes and lactoperoxidase are present. It may improve our mechanism of defense by improving the immune system if it is consumed on daily basis. The amount of sugar and cholesterol is very low, which is why it is considered superior to all other ruminant milk. It has a miraculous impact on the health of human beings as it contains insulin, and vitamin C is present in a very high amount (12). The vitamins contained play an important role as it has antioxidant activity as well as a role in the regulation of damage caused by destructive substances (13). This problem was handled with scientific research in which the antioxidant activity was determined to prove the remedial properties of camel milk (14).

The whey proteins, casein proteins, and lactic acid bacteria from the camel milk were evaluated to determine its antioxidant activity; regulation of the immune system, role in inflammation, activities related to probiotic properties, and anti-microbial were studied (16–19). Table 2 elaborated the mechanism of action behind such bioactive components of camel milk in the body against various biomarkers. Because of its antioxidant capability, camel milk is used to remove the side effects caused by chemo- and radiotherapy, as well as being used in the treatment of many types of cancers (20). Studies have shown that camel milk produces important beneficial effects on human health, as it contains low levels of β-lactoglobulin, making it suitable for consumption by persons who cannot tolerate lactose in their meals (21).

TABLE 1 The nutritional components of camel milk.

Nutrients in camel milk	Values	References
Lactose	49.8 g/l	(15)
Fat	35.6 g/l	
Polyphenols	35.45 mg GAE/1	
Protein	32.6 g/l	
Flavonoids	29.05 mg EQ/1	
Vitamin C	27.53 mg/l	
Ashes	8.06 g/l	

The popularity of camel milk is due to its source nutrient content. In some areas of Africa and Asia, it is consumed as a major part of their staple diet and it is assumed that it may have a role in the promotion of good health. For this reason, the utilization of camel milk in the form of fresh milk or sour milk is recommended so that the complications of liver or kidney disease such as increased oxidative stress, delayed wound healing, and high levels of cholesterol in the blood can be controlled. Therefore, an effort is made here to compile the latest available literature from the last 10 years studies were done on the health promotion and protectiveness of camel milk toward liver disorders of various kinds.

Liver disorders

In the functioning of the human body, the liver plays a vital role. It is responsible for detoxification and it metabolizes various components of food that enters our body (34). It is involved in the metabolic, vascular, secretory, immunological, and excretory functions of the human body. The metabolism of key nutrients such as carbohydrates, proteins, and fats is also served by the liver (35). The control of the flow of substances that are absorbed from the digestive system and then distributed to blood circulation to play its key role in the targeted organ site is the primary function of the liver. The total loss in its functions can cause sudden death which shows its great importance in the human body (36). So, it is very important to keep the liver healthy, otherwise, it may cause several fatal diseases of the liver including liver cirrhosis, hepatitis, fatty liver disease, alcoholic liver disease, etc. are included (37).

Globally, hepatic diseases are a major human health problem, in which the rates of morbidity and mortality are high. Many things play role in the cause of injury to the liver such as alcohol, drugs, viruses, and lipopolysaccharides type

TABLE 2 Mechanism of action of bioactive components of camel milk.

Active component	Mechanism	References
Lactoferrin	• Monocytes or macrophages and granulocytes are upregulated • Vital function during the early phases of viral infection • Takes over the antiviral response • Improves the level of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) biomarkers	(22, 23)
Whey protein concentrate	• Minimizes the effect of viral load reducing • Serum albumin normalized • Improvement in the neutrophils' phagocytic function • Serum levels of alanine aminotransferase (ALT), ICAM-1, IL-2 reduced	(24)
Immunoglobulin	• Ability to enter ins tissues and cells • The enzyme activity of bacteria or viruses is normalized	(25)
Ascorbic acid	• Helps in improving liver function	(26)
Vitamin B, C, and E	• Act as an antioxidant • Plays a role in the reduction of hepatic fat accumulation • Oxidative stress of systemic and hepatic systems is reduced	(27)
Low lipid content	• The high value of L-carnitine • Absorption of cholesterol decreases	(28)
Camel milk proteins	• Protection of non-alcoholic fatty liver diseases • Reduces inflammatory angiogenesis	(29, 30)
Lactoferrin	• Inhibitory effect on HCV • In chronic hepatitis C patients, lactoferrin improves HCV RNA and ALT levels	(31)
Magnesium	• Delays the aging process of skin • Major role in the hair growth	(32)
Zinc	• Alcoholic liver disease is improved *via* processes like • Programmed death of hepatocytes • Reduction of endotoxemia • Proinflammatory cytokines decreased	(33)

of bacteria (38). There are different mechanisms as well as many risk factors present that have a role in the expansion of healthy liver into liver fibrosis, cirrhosis, failure of the liver, and the many related complications, which in some cases progress to liver cancer. There are many toxins taken into the liver, such as high intake of alcohol, heavy metals, and organic and inorganic solvents, and when exposed these can lead to the production of many free radicals in the liver that may develop into hepatic lesions, which include liver cirrhosis, hepatitis, and liver carcinoma (39). The exposure of the liver to radiation may be accidental or therapeutic, but the damage will lead to the improper functioning of the liver which may include the excretion of harmful waste products, production of bile, storage of glucose in the form of glycogen, and synthesis of protein (40). There are multiple diseases or problems of the liver, for example, hepatitis, alcoholic liver disease, hepatotoxicity, liver carcinoma, cirrhosis, etc. Therefore, the effect of camel milk on these hepatic diseases has been discussed one by one to highlight the liver protective potential.

Hepatoprotective effect of camel milk

Table 3 explains the scientific studies reporting the hepatoprotectiveness of camel milk. One such study was done to determine the impact of camel milk on the enzymes of the liver, total proteins, and histology of poloxamer 407 induced hyperlipidemic rats when it is orally given to the Wister rats having a hyperlipidemic problem. Thirty male Wister rats whose weight was between 150 and 200 g were divided into six groups having five rats each. To evaluate the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin value, globulin, the ratio of albumin and globulin, samples of rats' blood and liver tissues were taken after 3 weeks. All the groups which were treated with camel milk showed a substantial ($p < 0.05$) decrease in the levels of ALT and AST. There is a markable reduction in the levels of total protein content, and globulin in the groups which are given with the camel milk at 250 and 1,000

TABLE 3 Scientific studies explain the hepatoprotectiveness of camel milk.

Year	Camel milk dosage	Subject	Type of liver disease	Materials and methods	Results	References
2022	Camel milk and camel urine	24 Mice divided into 4 groups	Hepatotoxicity	G1 = control, G2 = positive CCL_4, G3 = camel milk (100 ml/day/cage) injected with CCL_4, G4 = camel Urine (100 ml/day/cage) injected with CCL_4	There is a defensive function of camel milk and camel urine against hepatotoxicity induced with CCL_4	(42)
2021	Camel's milk	Adult female Sprague Dawley rats = 100	Hepatotoxicity	G1 = Oral dose of MXC 200 mg/kg BW (methoxychlor-induced liver damage), G2= (100 mL/day) camel milk given for 6 or 12 months, G3: daily dose of (100 mL/day) for 6 or 12 months	There is protecting role of camel milk against methoxychlor-induced liver damage	(43)
2021	Probiotics from camel milk	Mice = 40	Liver injury	Model groups = skimmed camel milk, Metformin group= 0.3 g per kg BW metformin. Probiotic groups= probiotics from camel milk are given in a low and high dose	The liver and kidney damage is improved with camel milk probiotics that regulate lipid metabolism, and protection in mice	(44)
2021	Camel whey protein hydrolysates (CWPH)	TAA- toxicity induced male Wistar albino rats=35	Hepatorenal failure	G1 = 5 mL sterile distilled water; G2 = TAA (200 mg/kg BW), G3 = TAA (200 mg/kg BW) + CWPH (50 mg/kg BW/day orally, G4 = TAA (200 mg/kg BW) + CWPH (100 mg/kg BW/day orally, G5 = TAA (200 mg/kg BW) + CWPH 200 mg/kg BW/day orally	CWPH has hepatorenal protective properties	(45)
2021	Camel milk antibodies	Male Wister rats	Hepatocellular carcinoma	Hepatocarcinoma induced by DENA + CCl_4 Then camel milk antibodies CM-IgG (100 mg/kg, orally) given	IgG from camel milk in the removal of dysfunction of liver cells oxidative stress induced by DENA	(46)
2021	Camel milk	Albino rats =96	Hepatotoxicity	G1: saline solution, All remaining groups: camel milk 2, 4, and 6 ml/100 g BW, respectively	Camel milk ingestion resulted in restorations of functions of kidney and liver biomarkers	(11)
2020	Camel milk	Mice = 24	Alcoholic liver disease	G1 (control group) = normal diet + 0.3 mL water, G2 (ethanol group) =normal diet + 0.3 mL water, G3 (Camel milk treatment group) = ethanol + camel milk and skimmed camel milk powder	Camel milk protects against liver injury caused by alcohol	(47)
2019	Camel milk lactoferrin	Male Sprague Dawley rats = 75	Hepatic fibrosis	CCL_4+ 40% CCL_4 (mix with olive oil) at 200 uL/100 g BW. Among all groups 30, 60, and 90 mg/kg/BW given with standard diet + lactoferrin orally Control group = standard diet throughout the study	Camel milk lactoferrin improved blood levels of ALP, AST, bilirubin, serum urea, and serum creatinine levels	(48)
2019	LAB from camel milk	Mice	Liver disease	Mice were given six strains of LAB for 7 weeks	Probiotics from camel act as a liver injury inhibitor	(38)

(Continued)

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TABLE 3 (Continued)

Year	Camel milk dosage	Subject	Type of liver disease	Materials and methods	Results	References
2018	Camel milk + NSO	Female albino Wister rats=30	Hepatotoxicity	G1 = normal control, G2 = toxic control, G3, G4, and G5 = camel milk, NSO, and NSO+ camel milk, respectively. Group VI = Unani medicine Jigreen	Protective effects of camel milk, and camel milk + *Nigella sativa* oil on the toxicity of liver and kidney in rats	(49)
2018	FCM	Male rats = 42	Non-alcoholic fatty liver disease	G1 = standard diet, G2 = HFDHFr to induce fatty liver disease The remaining five groups = HFDHFr + camel milk, (FCM) having non-encapsulated probiotic bacteria, FCM having microencapsulated probiotic without prebiotic, FCM containing microencapsulated probiotic and 1% ginger extract or FCM having microencapsulated probiotic and 10% beetroot extract, respectively	FCM containing microencapsulated probiotics with plant extract reduced the severity of fatty liver	(50)
2018	FCM	Female Wister mice = 56	Liver damage	Control mice= water+ standard diet G2: CCL_4 in 0.3% olive oil, G3: FCM, G4: *R. officinalis,* G5 = *R. officinalis* + FCM, G6: firstly given with FCM then toxicated with $CCL_{4,}$ G7: Initially treated with *R. officinalis* then toxicated with $CCL_{4,}$ G8: Initially treated with FCM+ rosemary then toxicated with CCL_4	FCM in combination + with *R. officinalis* extract is beneficial in reducing liver injury	(51)
2018	FCM	Human (adults)	Liver enzymes status	Overweight/obese adolescents were given camel milk 250 cc per day for 8 weeks, then diluted Cow milk yogurt 250 cc per day for 8 weeks or vice versa	FCM can be given as a functional food supplement	(52)
2018	Camel milk	Rats = 30	Hepatotoxicity	G1 = 0.5 ml normal saline, G2 = 3 g/kg/day ethanol, G3 = 1 mL/kg/day/orally camel milk, G4 = ethanol (3 g/kg/day) + camel milk (1 mL/kg/day), G5 = ethanol (3 g/kg/day) group	Camel milk has a protective and prophylactic effect against liver toxicity induced by ethanol	(53)
2017	Camel milk yogurt enriched with fig and honey	Male albino rats = 47	Steatohepatitis	G1 = +ve control MCDD, G2 = MCDD + Camel milk yogurt 30%, G3, G4, and G5 were given MCDD with 30% camel milk yogurt+ fig and honey, respectively	Protective effect on steatohepatitis	(54)

(Continued)

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TABLE 3 (Continued)

Year	Camel milk dosage	Subject	Type of liver disease	Materials and methods	Results	References
2017	Camel milk+ EVOO	Mice	Liver toxicity	G1 = Acetaminophen (500 mg/kg), G2 = camel milk (33 ml/kg), G3 = extra virgin olive oil (1.7 ml/kg), G4 = acetaminophen (500 mg/kg), G5 = camel milk +acetaminophen	Olive oil and camel milk have hepatoprotective action	(55)
2017	Camel milk given with drug cisplatin	Male rats = 56	Hepatocarcinogenesis	G1 = control group, G5, G6, G7, and G8 = DENA (200 mg/kg BW) and phenobarbitone (500 ppm) in drinking water, G2, G3, G4, G7, and G8 = Camel milk (5 mL/day) and cisplatin (5 mg/kg/3 weeks)	Reduction in the hepatocarcinogenesis with camel milk intake	(56)
2017	Camel milk + Peg IFN/RBV)	Human (adult patients) = 45	Chronic hepatitis C	G1 = (n = 23) Peg IFN/RBV in standard-dose, while G2 = (n = 22) Camel milk+ Peg IFN/RBV	Camel milk + Peg IFN/RBV improve the viral response + harmful effects of chronic hepatitis C are reduced	(57)
2017	Camel milk	Human= 17 patients (12 male + 5 females	Hepatitis C	Control = Three healthy adults included in study Participants = routine daily meals + camel milk 250 ml for 4 months	Camel milk decreased the viral load in the patient's sera	(58)
2017	Camel milk	Male Wister rats = 30	Altered liver enzymes	G1 = distilled water, G2 = induced with P407, G3 = induced with P407 then given atorvastatin (20 mg/kg), G4,5,6 = induced with P407 then camel milk 250, 500, and 1,000 mg/kg	Hepatoprotective effect of camel milk	(41)
2017	Camel milk in anti-tuberculous drugs	Male albino rats = 24	Hepatotoxicity	Rats were given a standard diet+ anti-tuberculous drugs+ camel milk G1 = normal diet + freshwater, G2 = anti-tuberculous drugs, G3 = 1 ml/kg BW of camel milk, G4 = 1 ml/kg BW of camel milk + Anti-tuberculous drugs	The toxicity and damage to the liver caused by anti-tuberculous drugs will be decreased with camel milk	(59)
2016	Camel milk + bee honey	Male rats = 36	Liver cirrhosis	G1 (control) = basal diet + tap water, G2 = basal diet + water intoxicated with CCL4, G3 = basal diet + camel milk, G4 = basal diet + camel milk + bee honey	Protecting effect of camel milk against CCL_4-induced liver damage.	(60)
2016	Camel milk	Male adult Rats = 24	Liver injury	G1 = corn oil, G2 = water + CCL_4 in a dose of 1 ml/kg in 50% corn oil, G3 = camel milk + corn oil, G4 = camel's milk+ CCL_4 in a dose of 1 ml/kg 50% in corn oil.	Camel milk protects the liver and kidney against CCL_{4-}generated oxidative stress and injuries	(61)

FCM, fermented camel milk; HFDHFr, high-fat diet and high fructose in water; Peg IFN/RBV, pegylated interferon and ribavirin; EVOO, extra virgin olive oil; LAB, lactic acid bacteria; NSO, nigella sativa oil; MCC, methionine choline-deficient diet; DENA, diethyl-nitrosamine.

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mg/kg, and there is an increase in the Albumin/Globulin ratio in all the groups that are treated with camel milk (41).

There is a key role of lactic acid bacteria could play in the pathogenesis of liver diseases. In this study, the isolation of 107 strains of lactic acid bacteria from the products of Mongolian camel milk was done which was then identified as species, and the screening was performed to determine their probiotic properties. To study the protective effects of these strains on the acute injury of the liver that is induced by lipopolysaccharide (LPS)/D-galactosamine (D-GalN), six strains of lactic acid bacteria, which have strong bearing and bounding capacity were isolated. For 7 weeks, these six strains of LAB were given to the rats. The amount of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) secretion in serum and liver, as well as the evaluation of expression of tumor necrosis factor (TNF) and interleukin (IL)-6 in the liver and intestines. This suggests the strength and role of probiotic and pharmacological value of *L. paracasei* subsp. In inflammation-based liver disease, paracasei is the inhibitor of liver injury (30).

In another study, rats injected with carbon tetrachloride (CCl4) showed upregulation of the expression of mRNA of hepatic IL-6 and renal IL-1β, TGF-β1, SREBP-1c, and caspase-6 and down-regulating the expressions of enzymes of anti-oxidation such as SOD, GST, and CAT in addition to hepatocellular vacuolation, mononuclear cell infiltration, and sinusoidal dilatation and renal glomerular atrophy, capsular space expansion, and adhesion between visceral and parietal layers of Bowman's capsule. Camel milk supplementation prior to and with CCL_4 injection to rats attenuated CCL4-induced hepatic and renal inflammatory cytokines (IL-6, IL-1β, TGF- β1 SREBP-1c, and caspase-6), upregulated CCL4-suppressed anti-oxidative markers (SOD, GST, and CAT) and induced protective and regenerative mechanism (EPO and IL-10). Additionally, camel milk protects the liver and kidney from CCL4-induced histopathological changes in vacuolation. These results showed the mechanism of camel milk protection of the liver and kidney against CCL_4-generated oxidative stress and injuries. The results of this study conclude the beneficial role of camel milk as a therapeutic adjuvant with drugs that are always associated with the production of oxidative stress that injured the liver and kidneys as anti-tumor drugs such as Cisplatin (62). Figure 1 explains the effect of camel milk in combination with drug treatment.

Likewise, a study was conducted to examine the effect of whey protein hydrolysates (WPH) of camel milk on the liver toxicity induced by thioacetamide (TAA) in rats. The assessment of enzymes of liver, protein and lipid profile, activities of antioxidant enzymes, function related to kidney, and pathological alterations in the liver was performed. There is a reduction in the antioxidant capacity, impaired functions related to liver and renal, and increased lipid content in blood in the animals having TAA toxicity. The CWPH conserved the hepatorenal functions, activities of antioxidative enzymes, and the lipid profile of the animals that are treated with CWPH, as well as countered the oxidative tissue damage caused by TAA. In addition, the CWPH can counteract the dysfunction of the hepatorenal. It showed that the camel whey protein has antihypertensive, antioxidant, and protective properties of hepatorenal after being hydrolyzed (37).

Moreover, a study was conducted in which the adolescents who are overweight or obese having complained of metabolic syndrome were given camel milk 250 cc per day for 8 weeks, then after 4 weeks of the gap, they were given diluted cow yogurt for the same time. After each period, the liver enzymes, anthropometric measurements, and serum lipids were assessed. Before each period, a food record of 3 days and a questionnaire related to physical activity were completed. Twenty-four participants of which 58% were girls, completed the study. In comparison to diluted cow yogurt, the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the ratio of AST/ALT are decreased with the intake of fermented camel milk. The study infers that FCM may be used as a dietary supplement after observing its favorable effects in the related condition (44).

In another study, composite probiotics from camel milk (CPCM) were prepared after the separation of 4 lactobacilli and 1 saccharomycetes from traditional fermented cheese whey (TFCW). Then its effects on the metabolism of glucose and lipid, hepatorenal functions, and gut microbiota in mice were investigated. Free access to food and water was given to each mouse and disinfected-skimmed camel milk was provided to the model group, the metformin in the amount of 0.3 g per kg of BW was given to the metformin group. A low and high-dose CPCM was provided to the low and high probiotic groups. Histological examination of the pancreas and liver was completed with the help of optical microscopy on paraffin material. The tissue sections of the pancreas and liver were fixed with a 10% buffered solution of formalin and then histological preparations were made. In addition, it was observed that there is a regulation in the metabolism of the liver, improvement in the functions of fatty liver, renal, and gut microbiota functions with the help of composite probiotics in camel milk that acts by regulating the intestinal flora disturbance and protecting the functions of islets (36).

Hepatitis

Hepatitis C virus (HCV) causes damage to the liver and is known as the major cause of it. Hepatitis is with medical therapies, though traditional and herbal medicines are also used. The prevalence of hepatitis globally is around 2.2%. In less developed countries like Pakistan, the rate of infection with hepatitis C grades on the second number ranging from in occurrence from 4.5 to 8% (63). It is the main cause of hepatic damage in developing countries such as Egypt as well,

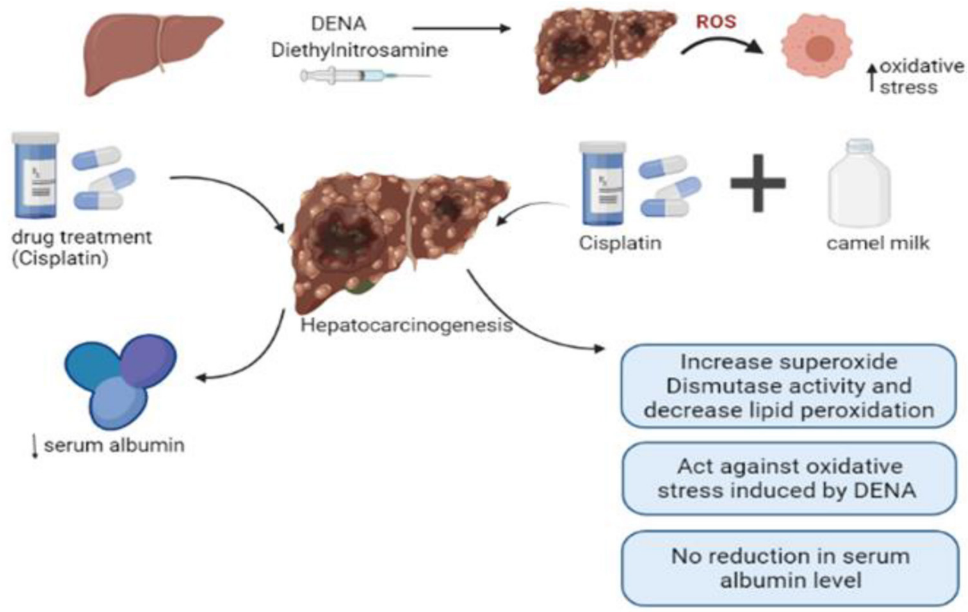

FIGURE 1
Effect of camel milk in combination with drug treatment.

and globally it is considered a major health issue. Worldwide, around 130–180 million people are infected with HCV. It has been suggested that in the upcoming 20 years, the rates of mortality due to hepatitis will continuously increase. The difference between acute infection and chronic infection of HCV is that there is the presence of jaundice as its symptom, there is a history of an increase in the levels of ALT, and the duration of an increase in ALT. RNA might be spotted after 2 weeks of exposure to HCV in acute infections, while the antibodies against HCV can be detected after 2–3 months of virus exposure (50). The objective of this review is to assess the role of camel milk on multiple disorders of the liver.

In another study, the impact of camel milk to treat hepatitis C patients was investigated. To determine the liver functions of the patients, a half-liter of fresh pasteurized camel milk was given to each patient on alternate days. The study concluded that camel milk has a positive effect on the patients as it improves their total protein, albumin, and lymphocyte levels (63). In another study, camel milk's effectiveness in the treatment of patients with hepatitis C was determined. The serum of patients was collected before and after drinking camel milk and to evaluate the serum, three biomarkers ALT, AST, and anti HCV antibodies profile were observed. The results showed that the majority of patients have a positive effect on camel milk as the levels of AST and ALT were reduced after 4 months of drinking camel milk (58).

In another study, proteins in camel milk, i.e., camel polyclonal IgGs, and α-lactalbumin were separated and the antiviral activity against HCV was observed with the use of PBMCs and Huh 7.5 cell lines. The incubation of HCV with the purified proteins was done directly or the incubation of proteins with the cells was done before the exposure to HCV. The entry of HCV was inhibited by the interaction of HCV with camel IgGs and lactoferrin. The camel milk lactose Ferrin can inhibit the replication of HCV in cells at a specified amount. The consequences of this study conclude that the infectivity of HCV was repressed by lactoferrin in camel milk (64). In another study, lactoferrin was considered the primary pharmaceutical drug against HCV infection. The virus entry was inhibited with the direct interaction of camel lactoferrin and HCV after 7 days of incubation. Thus, the results conclude that lactoferrin has antiviral activity and an inhibitory role in the pathway of HCV infection and has more effectiveness than human lactoferrin (23).

Hepatocellular carcinoma

The prime cancer of the liver is Hepatocellular carcinoma (HCC), worldwide, it is considered the fifth most common cancer and the third leading cause of mortality due to cancer. Its occurrence is common in many areas of the world as Asia, sub-Saharan Africa, as well as parts of Europe and the North American continent. In Egypt, the incidence rate of HCC in patients with liver diseases has increased over the past 10 years. In the occurrence of

HCC, many risk factors such as inflammation have been implicated (65).

The beneficial potential of camel milk can be enhanced with the addition of cisplatin. The attribute of this positive therapeutic effect was due to the increase in the activity of superoxide dismutase and decrease in lipid peroxidation as well as a drop in the mean area of changed hepatocellular foci and the mean area of P-GST positive foci. The antioxidant effect of camel milk may have a role in the reduction of hepatocarcinogenesis when given cisplatin (56). In another study, El-Miniawy determined the impact of camel milk on the rats which were induced with hepatocarcinogenesis. The 28 rats were grouped into four groups having seven rats each. Diethyl-nitrosamine (DENA) injection was given to rats in the 6th week of camel milk treatment and after 34 weeks of injection, three rats were sacrificed from each group. The remaining rats were sacrificed on the 9th week of camel milk treatment after week 38. The levels of AST, ALT, albumin, and total protein in serum were examined spectrophotometrically. The levels of AST, ALT, albumin, total protein, and alpha-fetoprotein (AFP) in the serum of rats who were sacrificed were evaluated. Then the liver was examined histo-pathologically and alpha-fetoprotein was stained immunohistochemically and glutathione s transferase of the placental liver was carried out. Camel milk has a role in the liver protection against toxicity which was induced by DENA, as well as the progression to hepatocellular carcinoma was prevented, and the hepatocellular carcinoma growth was stopped (65).

Moreover, the beneficial antitumor effects of antibodies in camel (*C. dromedarius*) milk (IgG) on DENA-induced carcinoma in liver cells in male Wistar rats were determined. Hepatocarcinoma was induced in rats using DENA (50 mg/kg, twice/week) for 2 weeks followed by CCl4 (1 ml/kg, trice/week) for 6 weeks. On week 17th, HCC-bearing rats were orally administrated camel milk IgG (100 mg/kg, orally) daily for 4 weeks. Liver enzyme activities and levels of alpha-fetoprotein (AFP) were measured in serum. Lipid peroxidation, and nitric oxide, reduced the levels of glutathione (GSH) were decreased, and the activity of superoxide dismutase (SOD) was determined in liver homogenate. Histological analysis using hematoxylin and eosin stain was examined in liver tissues. Hepatic mRNA gene expression of placental glutathione-s-transferase (GST-P) was determined by qRT-PCR. Treatment of HCC-bearing rats with IgG of camel milk significantly reduced liver injury biomarkers and attenuated oxidative stress as well as enhanced antioxidant status. Moreover, IgG camel milk significantly alleviated hepatocellular morphology alterations and down-regulated GST-P gene expression levels in the liver. The study concluded that there is an improvement in the dysfunction of the liver induced by DENA as well as in the oxidative stress in the rats having hepatocarcinoma was all attributed to the immunoglobulins purified from camel milk (46). Figure 2 explains the effect of camel milk on liver carcinoma.

Alcoholic/non-alcoholic liver disease

Alcoholic liver disease (ALD) is a major cause of the increased number of disease cases and death rate worldwide. In Europe and the United States, ALD is considered one of the most common liver diseases, and ∼300 million people are affected by hepatitis B virus, NAFLD, and ALD in China. ALD is characterized by a wide range of morphological features such as fatty liver—also known as steatosis—hepatitis, and alcoholic cirrhosis (39).

It is considered one of the major problems of liver diseases, and it is characterized by an imbalance of the intestinal microbes and hepatic inflammation. In the study, the rats having acute ALD were taken, and then the hepatoprotective effects of camel milk were investigated. Camel milk was given to different groups to determine the effect on the biochemical indicators. The hepatic inflammation in the ethanol plus camel milk group showed a reduction when the serum biochemical indexes and histology were analyzed. Camel milk suppresses the expression of genes related to inflammation such as (ILB and CXCL1) in the IL-17 and tumor necrosis factor (TNF-α) pathways. The results infer that hepatic inflammation and disorder in the microbial intestine, which were caused by acute alcohol injury were modulated with camel milk, which indicates the defending role of camel milk against liver injury induced by alcohol (39).

Moreover, the progression of NAFLD which was induced by a diet rich in fat and high fructose water was given to rats, and the preventive effects of FCM were investigated. Seven different groups were made and 42 male rats were divided into these groups randomly. A high-fat diet and high fructose in water (HFDHFr) were used to induce fatty liver disease. Camel milk was given as FCM or camel milk containing probiotic bacteria or containing probiotic without prebiotic, camel milk with 1% ginger extract, or microencapsulated probiotic with 10% beetroot extract. The serum of rats was analyzed to infer the activity of liver enzymes, insulin resistance, lipid profile, inflammation markers, and the stress due to oxidation. The study concluded that the concentration of serum glucose and the activity of liver enzymes were reduced with the intake of FCM-containing probiotics. Moreover, the groups that were given FCM containing probiotics and beetroot extract and camel milk with ginger extract have a positive impact on the liver (42).

Another study was conducted on 30 male and female rats to evaluate the reactivation of liver functions due to the camel milk in the rats damaged by the Sudanese liquor (Aragi). The 24 rats were divided equally into two groups, control and tested. The levels of Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT), and Alkaline Phosphatase (ALP) were measured for both groups. Liver samples were investigated and it infer that camel milk reduced the level of these enzymes to their normal. The reductions in the levels of these enzymes from day (30) to day (60) were 73.2, 53.9, and 65.4%, respectively. It was concluded that camel milk can

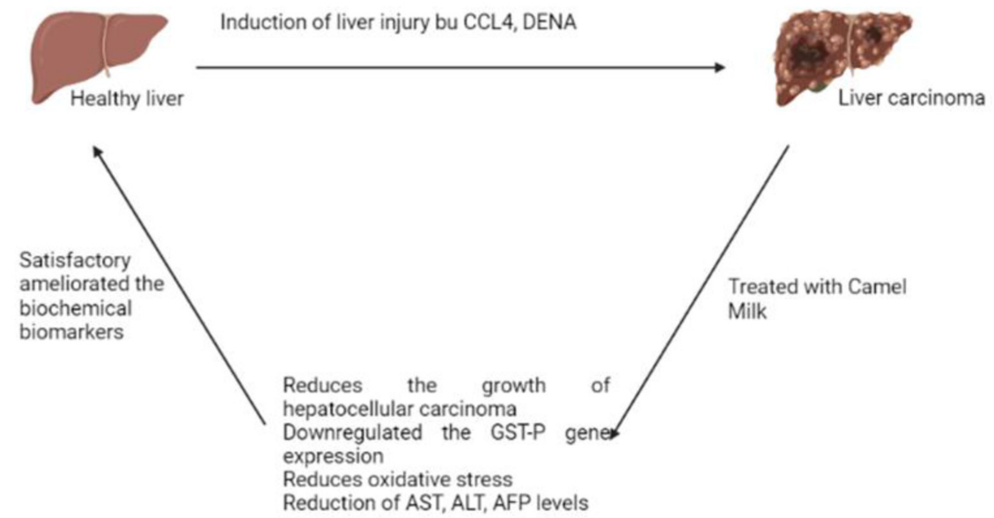

FIGURE 2
Effect of camel milk on liver carcinoma.

be used as a herbal treatment for alcoholic diseases as well as in the treatment of other liver diseases which may have an impact on the liver enzymes and other tissues (66). Figure 3 presents the effect of camel milk on alcoholic liver disease.

Hepato-toxicity

To assess how camel milk impacts newborn rats who are given ethanol to induce hepatotoxicity, a study was conducted. The rats were divided into five groups having six rats in each group. There is a significant rise in the levels of MDA and the activity of SOD, CAT, and GSH-PX enzymes were reduced in the liver tissues of newborn rats as well as there are histological changes in the tissues of the liver when they are treated with ethanol during pregnancy. Moreover, when this group was related to the control group the activity of serum enzymes was increased. The study concludes that there are protective and preventative effects of camel milk on newborn rats who are induced with hepatotoxicity (45). In another study, the toxic properties of CCL_4 on the tissue of the liver were examined with exposure to camel milk and camel urine. Liver enzymes including ALT, AST, and ALP were monitored as there is an increase in their values when a single dose of CCL_4 was given to them. The four groups were made and rats were divided equally, and then each group was given a different diet the first group was the control group to which standard diet and tap water were provided. The second group was named as a positive control group and given 1 ml/kg of BW of CCL_4 with the same ratio of 1:1 of olive oil for 4 weeks. The third group was given camel milk (100 mL/day) with a normal diet and CCL4 (1 ml/kg of BW) was injected and the hepatoprotective effect was tested. The fourth group was fed with camel urine (100 mL/day) with a normal diet and CCL_4 (1 ml/kg of BW) was injected. The blood samples of rats were collected and analyzed. The serum was separated by centrifugation and the serum activities of ALP, AST, and ALT were monitored to detect the hepatotoxicity caused by CCL_4. The study concluded that the group which was given camel milk had a positive effect in improving the hepatotoxicity caused by CCL_4. The study also concludes that camel urine has a protective effect against toxicity (34).

Moreover, camel milk contains lactoferrin which has antiviral, as well as anti-inflammatory properties. It can be utilized to treat hepatic fibrosis in Sprague Dawley rats caused by CCL4. Five groups were made by dividing 75 male Sprague–Dawley rats randomly. Carbon tetrachloride (CCL_4) was provided to each group at 200 uL/100 g BW as a single dose with a mixture of 40% CCL_4. The standard diet was provided to the control group while the remaining four groups were provided orally with lactoferrin along with the standard diet. It was a two phase study in which in the initial phase, the camel milk lactoferrin was isolated and purified. In the next phase, lactoferrin's efficacy against carbon tetrachloride-induced liver toxicity in Sprague Dawley rats was investigated. The study concludes that lactoferrin in camel milk may cause improvements in the liver toxicity induced by CCL_4 in rats illuminates that there are improvements in the serum levels of ALP, AST, AST, bilirubin, serum urea, and serum creatinine in 4 weeks of treatments with camel milk lactoferrin (40).

Similarly, Camel milk and *Nigella sativa* oil (NSO) have anti-hepatotoxic potential against hepatic and nephrotoxicity induced by TAA in rats was investigated. The study was

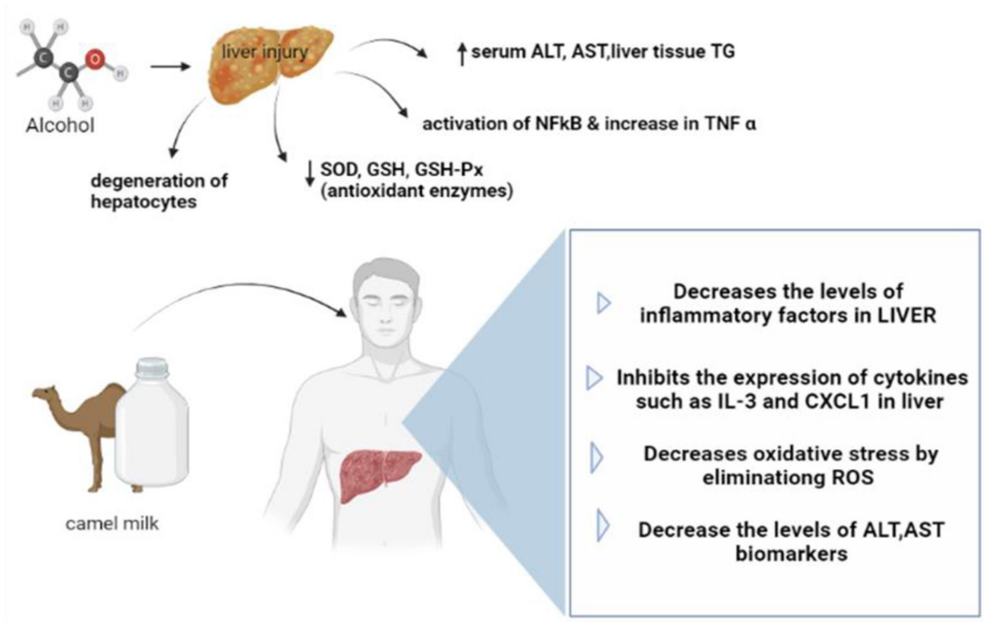

FIGURE 3
Effect of camel milk on alcoholic liver disease.

conducted on rats and six groups were made of 30 female rats that were divided equally into these groups having five rats in each group. To induce hepatorenal toxicity, on the first day, all animals from Groups II to VI received a single injection of TAA (100 mg/kg BW) in the form of a 2% w/v solution. The histopathological investigations were done on the preserved tissues of the liver and kidney. Commercial diagnostic kits were used to determine biochemical parameters such as serum levels of AST, ALT, GGT, ALP, uric acid, urea, creatinine, salt, and potassium. To check the changes, histopathological assessments of liver and kidney tissues were assessed according to the standard method. In comparison to the normal control rats, a single injection of TAA (100 mg/kg) was administered by injection that will increase the blood levels of ALT, AST, ALP, and GGT, confirming the initiation of hepatotoxicity. When the experimental animals are treated with camel milk, NSO alone or its combination may increase the levels of AST, ALT, and ALP and improve the liver toxicity induced by TAA. The results concluded that camel milk, NSO, and camel milk in combination with NSO are effective in correcting toxicity of liver and kidney in rats (41).

Likewise, in gamma-irradiated Albino rats, the preventive properties of camel milk were examined. The 96 Sprague Dawly healthy adult male albino rats were classified into 16 groups and then labeling was done. The parameters of hepatological and nephrological were biochemically measured in the blood sample that was collected. The examinations of the liver and kidney were done. There will be changes in the functions of the liver and kidney along with changes in protein profiles caused by IRR. There will be dose-dependent reduction in the levels of serum protein such as total soluble proteins, and the levels of albumin and globulin. There will be an increase in the serum levels of bilirubin, urea, uric acid, ALT, AST, ALP, and creatinine levels significantly. Camel milk therapies in IRR rats restored their damaged status and there will be a reduction in the changes in liver and kidney functions, as well as hematological abnormalities related to IRR's adverse effects (3). Similarly, there is a restoration effect of camel milk on the liver toxicity induced by methoxychlor (MXC). Methoxychlor is an environmental contaminant, that is commonly used in many countries as a pesticide, and here it has been used for the induction of liver toxicity in rats. There is a significant increase in the levels of serum transaminases (AST and ALT) and alkaline phosphatase when MXC is given to rats, while there is a significant decrease in the levels of total protein and albumin. Lipid peroxidation is inhibited with MXC and it causes the elevation of glutathione levels in the liver homogenate. In the liver, pathological damages such as degradation of hepatic cells and coagulative necrosis were discovered. The daily dose of 100 ml/day in each cage of the camel milk-treated group was given and it was the only source of drinking for them for 6–12 months. On the other hand, an oral dose of MXC 200 l/kg of BW two times a week was given to the MXC-treated group, for the same period. The study concluded that there is a reduction in the deterioration of liver cells and normal structure of other cells, as well as the liver histopathological analysis, which was inconsistent with the biochemical findings. These effects proved the hepato-protective role of camel milk (35).

Cirrhosis

A study was conducted on male rats to investigate the impact of camel milk in combination with bee honey against hepatotoxic compounds. Two main groups were made and 36 rats were divided into these groups. The first group was the control group having $n = 9$ non-cirrhotic rats. The rats $n = 27$ in the other group were given carbon tetrachloride injection for the induction of cirrhosis. It was determined that the activities of enzymes of the liver, blood glucose level, non-esterified fatty acids (NEFA) in the serum, and glycogen amount in the liver increased with CCL4. Similarly, in the liver tissues, the activity of the phosphorylase enzyme is reduced, and elevated carbohydrate intolerance as well as the resistance index of insulin. Furthermore, CCL_4 has an elevated impact on the expression of TNF-α and TGF-β which are cytokine genes, and it induces the elevation of oxidative stress. However, camel milk can improve the toxic effects either alone or in combination with bee honey. This protection is based on the antioxidant capabilities of these preventive compounds and their impact on downregulating specific pro-cirrhotic cytokine gene transcripts (52).

In a study, Egyptian patients who are infected with HCV were enrolled having infection in the parenchyma which is mild to moderate and followed by mild cirrhosis after taking their history and clinical examination. To check the effect of camel milk, it is given to the patients, and then their biomarkers were evaluated. The marked inhibition of serum levels of the inflammatory biomarkers showed the improving effect of camel milk. The study concluded that camel milk has a regulatory function on the multiple parameters of mediators of inflammation, modulators of the immune system, antiapoptotic, and antioxidants, which infers the potential therapeutic advantage of camel milk against HCV (67).

Conclusion

The present study highlighted the protective and therapeutic role of camel milk on multiple liver disorders. People who consume camel milk have a significant improvement in the lab values of serum gamma-glutamyl transferase, aspartate aminotransferase, and alanine aminotransferase. The proteins in camel milk are heat-stable even at high temperatures and remain functional. It is therefore immensely important to understand the special attributes of camel milk and make it possible to fully utilize its potential. The prospect includes the utilization of camel milk as a nutraceutical agent and makes it possible to easily avail this in markets because it is a good choice for people who are lactose intolerant. So, the consumption of camel milk alone or in combination with any nutraceutical should be encouraged.

Author contributions

Conceptualization: KS, I-U-H, RR, and RA. Writing—original draft preparation: KS, RR, and SS. Writing—review and editing: AN, MM, NW, CS, CM, and RA. Supervision: RA. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Shabbir MA, Ahmed H, Maan AA, Rehman A, Afraz MT, Iqbal MW, et al. Effect of non-thermal processing techniques on pathogenic and spoilage microorganisms of milk and milk products. *Food Sci Technol.* (2020) 41:279–94. doi: 10.1590/fst.05820

2. Ahmad T, Butt MZ, Aadil RM, Inam-ur-Raheem M, Bekhit AED, Guimarães JT, et al. Impact of nonthermal processing on different milk enzymes. *Int J Dairy Technol.* (2019) 72:481–95. doi: 10.1111/1471-0307.12622

3. Zakaria A, Mohamed R. Effect of calf gender on milk composition, reproductive hormones and serum biochemical parameters of female dromedary camel. *Int J Vet Sci.* (2021) 10:47–50. doi: 10.47278/journal.ijvs/2020.004

4. Roshdy S, Omar L, Sayed R, Hassan H, Hanafy M, Soliman R. Reduction of milk contamination with aflatoxin-M1 through vaccination of dairy cattle with aflatoxin-B1 vaccine. *Int J Vet Sci.* (2020) 9:528–33. doi: 10.37422/IJVS/20.069

5. Ibrahim A, Saad M, Hafiz N. Toxic elements in dried milk and evaluation of their dietary intake in infant formula. *Int J Vet Sci.* (2020) 9:563–7. doi: 10.37422/IJVS/20.070

6. Balbin M, Lazaro J, Candelaria C, Cuasay J, Abes N, Mingala C. Evaluation of physico-chemical properties and nutrient components of dairy water Buffalo (*Bubalus bubalis*) milk collected during early lactation. *Int J Vet Sci.* (2020) 9:24–9. Available online at: http://www.ijvets.com/.../24-29.pdf

7. Desouky MM, Salama HH. Preparation and properties of children food after weaning using camels' milk and guadar cereal nanoparticles. *J Food Process Preserv.* (2021) 45:e15012. doi: 10.1111/jfpp.15012

8. Desouky MM, Salama HH, El-Sayed SM. The effects of camel milk powder on the stability and quality properties of processed cheese sauce. *Acta Sci Polon Technol Aliment.* (2019) 18:349–59. doi: 10.17306/J.AFS.0645

9. Zeid EHA, El Sharkawy NI, Moustafa GG, Anwer AM, Al Nady AG. The palliative effect of camel milk on hepatic CYP1A1 gene expression and DNA damage induced by fenpropathrin oral intoxication in male rats. *Ecotoxicol Environ Saf.* (2021) 207:111296. doi: 10.1016/j.ecoenv.2020.111296

10. Alebie G, Yohannes S, Worku A. Therapeutic applications of camel's milk and urine against cancer: current development efforts and future perspectives. *J Cancer Sci Ther.* (2017) 9:468–78. doi: 10.4172/1948-5956.1000461

11. Abdel-Mobdy AE, El Hussieny MS, Mobdy YEA. Evaluation of therapeutic and protective influences of camel milk against gamma radiation–induced hematotoxicity, hepatotoxicity and nephrotoxicity in albino rats. *Ann Roman Soc Cell Biol.* (2021) 25:7958–76. Available online at: https://www.annalsofrscb.ro/index.php/journal/article/view/2336/1955

12. Sharma A, Lavania M, Singh R, Lal B. Identification and probiotic potential of lactic acid bacteria from camel milk. *Saudi J Biol Sci.* (2021) 28:1622–32. doi: 10.1016/j.sjbs.2020.11.062

13. Al-Humaid A, Mousa H, El-Mergawi R, Abdel-Salam A. Chemical composition and antioxidant activity of dates and dates-camel-milk mixtures as a protective meal against lipid peroxidation in rats. *Am J Food Technol.* (2010) 5:22–30. doi: 10.3923/ajft.2010.22.30

14. Salami M, Moosavi-Movahedi AA, Moosavi-Movahedi F, Ehsani MR, Yousefi R, Farhadi M, et al. Biological activity of camel milk casein following enzymatic digestion. *J Dairy Res.* (2011) 78:471–8. doi: 10.1017/S0022029911000628

15. Bouhaddaoui S, Chabir R, Errachidi F, El Ghadraoui L, El Khalfi B, Benjelloun M, et al. Study of the biochemical biodiversity of camel milk. *Sci World J.* (2019) 2019:2517293. doi: 10.1155/2019/2517293

16. Yassin MH, Soliman MM, Mostafa SA-E, Ali HA-M. Antimicrobial effects of camel milk against some bacterial pathogens. *J Food Nutr Res.* (2015) 3:162–8. doi: 10.12691/jfnr-3-3-6

17. Homayouni-Tabrizi M, Asoodeh A, Soltani M. Cytotoxic and antioxidant capacity of camel milk peptides: effects of isolated peptide on superoxide dismutase and catalase gene expression. *J Food Drug Anal.* (2017) 25:567–75. doi: 10.1016/j.jfda.2016.10.014

18. Arab HH, Salama SA, Eid AH, Omar HA, Arafa E-SA, Maghrabi IA. Camel's milk ameliorates TNBS-induced colitis in rats *via* downregulation of inflammatory cytokines and oxidative stress. *Food Chem Toxicol.* (2014) 69:294–302. doi: 10.1016/j.fct.2014.04.032

19. Ibrahim HR, Isono H, Miyata T. Potential antioxidant bioactive peptides from camel milk proteins. *Anim Nutr.* (2018) 4:273–80. doi: 10.1016/j.aninu.2018.05.004

20. Aref A. Camel milk as a complementary and alternative medicine. *Int J Sci Res.* (2018) 7:435–9.

21. Cardoso RR, Santos R, Cardoso C, Carvalho M. Consumption of camel's milk by patients intolerant to lactose. A preliminary study. *Rev Aler Mexico.* (2010) 57:26–32. Available online at: https://bengreenfieldlife.com/wp-content/uploads/2017/02/Consumption-of-camel%E2%80%99s-milk-by-patients-intolerant-to-lactose.-A-preliminary-study.pdf

22. Legrand D, Elass E, Pierce A, Mazurier J. Lactoferrin and host defence: an overview of its immuno-modulating and anti-inflammatory properties. *Biometals.* (2004) 17:225–9. doi: 10.1023/B:BIOM.0000027696.48707.42

23. Iwasa M, Yamamoto M, Tanaka Y, Kaito M, Adachi Y. Spirulina-associated hepatotoxicity. *Am J Gastroenterol.* (2002) 97:3212. doi: 10.1111/j.1572-0241.2002.07145.x

24. Elattar EE, Goulermas J, Wu QH. Electric load forecasting based on locally weighted support vector regression. *IEEE Trans Syst Man Cybern Part C.* (2010) 40:438–47. doi: 10.1109/TSMCC.2010.2040176

25. Gul W, Farooq N, Anees D, Khan U, Rehan F. Camel milk: a boon to mankind. *Int J Res Stud Biosci.* (2015) 3:23–9. Available online at: https://bengreenfieldlife.com/wp-content/uploads/2017/02/Camel-Milk-A-Boon-to-Mankind.pdf

26. Farah Z, Rettenmaier R, Atkins D. Vitamin content of camel milk. *Int J Vitam Nutr Res.* (1992) 62:30–3.

27. Barbagallo M, Dominguez LJ, Tagliamonte MR, Resnick LM, Paolisso G. Effects of vitamin E and glutathione on glucose metabolism: role of magnesium. *Hypertension.* (1999) 34:1002–6. doi: 10.1161/01.HYP.34.4.1002

28. Alhomida A, Junaid M, Al-Jafari A. The distribution of total, free, short-chain acyl and long-chain acyl carnitine in ocular tissues of the camel (*Camelus dromedarius*). *J Ocul Pharmacol Therap.* (1997) 13:381–7. doi: 10.1089/jop.1997.13.381

29. Korish AA, Arafah MM. Camel milk ameliorates steatohepatitis, insulin resistance and lipid peroxidation in experimental non-alcoholic fatty liver disease. *BMC Complem Altern Med.* (2013) 13:264. doi: 10.1186/1472-6882-13-264

30. Alhaider AA, Abdel Gader AGM, Almeshaal N, Saraswati S. Camel milk inhibits inflammatory angiogenesis *via* downregulation of proangiogenic and proinflammatory cytokines in mice. *Apmis.* (2014) 122:599–607. doi: 10.1111/apm.12199

31. Redwan ERM, Tabll A. Camel lactoferrin markedly inhibits hepatitis C virus genotype 4 infection of human peripheral blood leukocytes. *J Immunoassay Immunochem.* (2007) 28:267–77. doi: 10.1080/15321810701454839

32. Patel A, Patel S, Patel N, Chaudhary G. Importance of camel milk-An alternative dairy food. *J Livestock Sci.* (2016) 7:19–25.

33. Husain H, Ahmad R. Role of zinc in liver pathology. In: *Microbial Biofertilizers and Micronutrient Availability.* Cham: Springer (2022). p. 101–13. doi: 10.1007/978-3-030-76609-2_5

34. Tufail S, Sehgal S, Niaz K. Hepatoprotective effect of camel milk in antituberculous drugs induced hepatotoxicity in male albino rats. *J Sheikh Zayed Med Coll.* (2017) 8:1260–4. Available online at: https://pesquisa.bvsalud.org/portal/resource/pt/emr-190496

35. Mitra V, Metcalf J. Metabolic functions of the liver. *Anaesth Intens Care Med.* (2009) 10:334–5. doi: 10.1016/j.mpaic.2009.03.011

36. Ozougwu JC. Physiology of the liver. *Int J Res Pharm Biosci.* (2017) 4:13–24.

37. Rabbi MF, Hasan SM, Champa AI, AsifZaman M, Hasan MK, editors. Prediction of liver disorders using machine learning algorithms: a comparative study. In: *2020 2nd International Conference on Advanced Information and Communication Technology (ICAICT)* (Dhaka) (2020). doi: 10.1109/ICAICT51780.2020.9333528

38. Xu R, Xiu L, Zhang Y, Du R, Wang X. Probiotic and hepatoprotective activity of lactobacillus isolated from Mongolian camel milk products. *Benef Microbes.* (2019) 10:699–710. doi: 10.3920/BM2018.0131

39. Nakagiri R, Hashizume E, Kayahashi S, Sakai Y, Kamiya T. Suppression by hydrangeae dulcis folium of D-galactosamine-induced liver injury *in vitro* and *in vivo*. *Biosci Biotechnol Biochem.* (2003) 67:2641–3. doi: 10.1271/bbb.67.2641

40. Alici-Karaca D, Akay B, Yay A, Suna P, Nalbantoglu OU, Karaboga D, et al. A new lightweight convolutional neural network for radiation-induced liver disease classification. *Biomed Signal Process Control.* (2022) 73:103463. doi: 10.1016/j.bspc.2021.103463

41. Zuberu J, Saleh MI, Alhassan AW, Adamu BY, Aliyu M, Iliya BT. Hepatoprotective effect of camel milk on poloxamer 407 induced hyperlipidaemic wistar rats. *Open Access Macedon J Med Sci.* (2017) 5:852. doi: 10.3889/oamjms.2017.158

42. Hussein M, Khan R. CCL4-induced hepatotoxicity: study in rats intoxicated with carbon tetrachloride and treated with camel milk and urine. *J Chem Stud.* (2022) 1:7–11. Available online at: https://al-kindipublisher.com/index.php/jcs/article/view/3039

43. Elsharkawy EE, Shaker EM, El-Nisr NA, Nahed MW. Methoxychlor hepatotoxicity and trials of camel milk restoration. *Asian Res J Curr Sci.* (2021) 3, 24–35.

44. Manaer T, Yu L, Nabi X-H, Dilidaxi D, Liu L, Sailike J. The beneficial effects of the composite probiotics from camel milk on glucose and lipid metabolism, liver and renal function and gut microbiota in db/db mice. *BMC Complem Med Ther.* (2021) 21:1–13. doi: 10.1186/s12906-021-03303-4

45. Osman A, El-Hadary A, Korish AA, AlNafea HM, Alhakbany MA, Awad AA, et al. Angiotensin-I converting enzyme inhibition and antioxidant activity of papain-hydrolyzed camel whey protein and its hepato-renal protective effects in thioacetamide-induced toxicity. *Foods.* (2021) 10:468. doi: 10.3390/foods10020468

46. Shawki AK, El-Desouky MA, Fouad SM, Ahmed AE-FM, Aboulhoda BE, Ahmed WA. Camel (*Camelus dromedarius*) milk antibodies ameliorated diethylnitrosamine-induced hepatocellular carcinoma in wistar rats. *Egypt J Chem.* (2021) 64. doi: 10.21608/ejchem.2021.65939.3417

47. Ming L, Qiao X, Yi L, Siren D, He J, Hai L, et al. Camel milk modulates ethanol-induced changes in the gut microbiome and transcriptome in a mouse model of acute alcoholic liver disease. *J Dairy Sci.* (2020) 103:3937–49. doi: 10.3168/jds.2019-17247

48. Asghar N, Nasir M, Iqbal S, Ahmad T, Majeed R. Effect of camel milk lactoferrin against carbon tetrachloride induced hepatic toxicity in Sprague Dawley rats. *Adv Life Sci.* (2019) 6:165–75. Available online at: https://www.als-journal.com/submission/index.php/ALS/article/view/797

49. Ahmad A, Al-Abbasi FA, Sadath S, Ali SS, Abuzinadah MF, Alhadrami HA, et al. Ameliorative effect of camel's milk and Nigella Sativa oil against thioacetamide-induced hepatorenal damage in rats. *Pharmacog Mag.* (2018) 14:27. doi: 10.4103/pm.pm_132_17

50. El-Abd MM, Abdel-Hamid M, Ahmed-Farid OA, El-demerdash ME, Mohamed ZF. protective effect of synbiotic fermented camel milk on non alcoholic fatty liver disease in rats. *Curr Sci Int.* (2018) 7:466–80. Available online at: https://www.curresweb.com/csi/csi/2018/466-480.pdf

51. Kamal H, Jafar S, Mudgil P, Murali C, Amin A, Maqsood S. Inhibitory properties of camel whey protein hydrolysates toward liver cancer cells, dipeptidyl peptidase-IV, and inflammation. *J Dairy Sci.* (2018) 101:8711–20. doi: 10.3168/jds.2018-14586

52. Fallah Z, Feizi A, Hashemipour M, Kelishadi R. Positive effect of fermented camel milk on liver enzymes of adolescents with metabolic syndrome: a double blind, randomized, cross-over trial. *Mater Soc Med.* (2018) 30:20. doi: 10.5455/msm.2018.30.20-25

53. Abbas MT, Ali AJ, Hamdan AA-A. The prophylactic-protective effect of camel milk on ethanol induced hepato-toxicity in newborn rats. *EurAsian J BioSci.* (2018) 12:503–9.

54. Rabeh NM. Effect of home made camel milk yoghurt supplemented with fig and honey bee in rats with induced steatohepatitis. مجلة بحوث التربية النوعية. (2017) 2017:533–58. doi: 10.21608/mbse.2017.138643

55. Ibrahim MAB, Wani FA, Rahiman S. Hepatoprotective effect of olive oil and camel milk on acetaminophen-induced liver toxicity in mice. *Int J Med Sci Publ Health.* (2017) 6:186–94. doi: 10.5455/ijmsph.2017.07092016614

56. El Miniawy HM, Ahmed KA, Mansour SA, Khattab MMS. *In vivo* antitumour potential of camel's milk against hepatocellular carcinoma in rats and its improvement of cisplatin renal side effects. *Pharm Biol.* (2017) 55:1513–20. doi: 10.1080/13880209.2017.1309553

57. al-Reza Hosseini SM, Zibaee S, Yousefi M, Taghipour A, Ghanaei O, Noras M. Camel milk with pegylated interferon Alfa-2a and ribavirin for treatment-naive chronic hepatitis C genotype 2/3: an open-label, randomized controlled trial. *Iranian Red Cresc Med J.* (2017) 19:e13529. doi: 10.5812/ircmj.13529

58. El-Fakharany EM, El-Baky NA, Linjawi MH, Aljaddawi AA, Saleem TH, Nassar AY, et al. Influence of camel milk on the hepatitis C virus burden of infected patients. *Exp Therap Med.* (2017) 13:1313–20. doi: 10.3892/etm.2017.4159

59. Tufail S, Sehgal SK, Niaz K. Hepatoprotective effect of camel milk in antituberculous drugs induced hepatotoxicity in male albino rats. *J Sheikh Zayed Med Coll.* (2017) 8:1260–4. Available online at: https://pesquisa.bvsalud.org/portal/resource/pt/emr-190496

60. Sadek K, Beltagy D, Saleh E, Abouelkhair R. Camel milk and bee honey regulate profibrotic cytokine gene transcripts in liver cirrhosis induced by carbon tetrachloride. *Can J Physiol Pharmacol.* (2016) 94:1141–50. doi: 10.1139/cjpp-2015-0596

61. Ibrahim ZS, Nassan MA, Soliman MM. Ameliorative effects of pomegranate on carbon tetrachloride hepatotoxicity in rats: a molecular and histopathological study. *Mol Med Rep.* (2016) 13:3653–60. doi: 10.3892/mmr.2016.4956

62. Ibrahim ZS, Alkafafy M, Soliman MM, Ahmed MM. Molecular mechanism of hepato-renal protection of camel milk against oxidative stress-perturbations. *J Camel Pract Res.* (2016) 23:53–63. doi: 10.5958/2277-8934.2016.00008.4

63. Sarfraz L. Effect of camel milk supplementation on blood parameters and liver function of hepatitis patients. *Am J Ethnomed.* (2014) 1:129–46.

64. El-Fakharany EM, Abedelbaky N, Haroun BM, Sánchez L, Redwan NA, Redwan EM. Anti-infectivity of camel polyclonal antibodies against hepatitis C virus in Huh7. 5 hepatoma. *Virol J.* (2012) 9:1–9. doi: 10.1186/1743-422X-9-201

65. El Miniawy HM, Ahmed KA, Tony MA, Mansour SA, Khattab MMS. Camel milk inhibits murine hepatic carcinogenesis, initiated by diethylnitrosamine and promoted by phenobarbitone. International *J Vet Sci Med.* (2014) 2:136–41. doi: 10.1016/j.ijvsm.2014.10.004

66. Ahmed AS, Abdalbagi N, Mustafa H, Idris A, Eltayb A, Ismail R. The role of camel milk in the reactivation of liver damaged by Sudanese liquor (Aragi). *J Public Health.* (2011) 6:157–63.

67. Mohamed WA, Schaalan MF, El-Abhar HS. Camel milk: potential utility as an adjunctive therapy to Peg-IFN/RBV in HCV-4 infected patients in Egypt. *Nutr Cancer.* (2015) 67:1307–15. doi: 10.1080/01635581.2015.1087041

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Insights into the constellating drivers of satiety impacting dietary patterns and lifestyle

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Food intake and body weight regulation are of special interest for meeting today's lifestyle essential requirements. Since balanced energy intake and expenditure are crucial for healthy living, high levels of energy intake are associated with obesity. Hence, regulation of energy intake occurs through short- and long-term signals as complex central and peripheral physiological signals control food intake. This work aims to explore and compile the main factors influencing satiating efficiency of foods by updating recent knowledge to point out new perspectives on the potential drivers of satiety interfering with food intake regulation. Human internal factors such as genetics, gender, age, nutritional status, gastrointestinal satiety signals, gut enzymes, gastric emptying rate, gut microbiota, individual behavioral response to foods, sleep and circadian rhythms are likely to be important in determining satiety. Besides, the external factors (environmental and behavioral) impacting satiety efficiency are highlighted. Based on mechanisms related to food consumption and dietary patterns several physical, physiological, and psychological factors affect satiety or satiation. A complex network of endocrine and neuroendocrine mechanisms controls the satiety pathways. In response to food intake and other behavioral cues, gut signals enable endocrine systems to target the brain. Intestinal and gastric signals interact with neural pathways in the central nervous system to halt eating or induce satiety. Moreover, complex food composition and structures result in considerable variation in satiety responses for different food groups. A better understanding of foods and factors impacting the efficiency of satiety could be helpful in making smart food choices and dietary recommendations for a healthy lifestyle based on updated scientific evidence.

KEYWORDS

appetite, food intake, food quality, diet, satiation, satiety

Introduction

The terms satiation and satiety are essential to understand the role of appetite in the regulation of food intake. Satiation is the feeling of fullness during an eating process, while satiety is the inhibition of hunger in response to eating (1). Hunger and satiety are involved in the maintenance of healthy body weight as energy intake and expenditure are mainly governed by the rate of gastric emptying as well as the metabolism of the nutrients. Energy balance is crucial to human survival and is dependent upon the amount of food consumed (2). Satiety not only determines the time elapsed between food ingestion at a meal and the next meal but also the prospective amount of food to be consumed at subsequent meals. The general population inherits the idea that foods with greater satiety are the ones that fill their stomach earlier. However, consumer perceptions are based on short-term satiety signals and orosensory learned indications. This area of consumer science is of particular interest in enhancing the knowledge and understanding of satiety perceptions among lay consumers (3).

The preparation and consumption of foods affect the mechanism of satiation and absorption of nutrients in the body. The feeling of hunger motivates the urge to eat. Thus, consuming food with superior satiating potential may help to achieve the desired dietary goals by decreasing overconsumption. From a nutritionist's perspective, satiety is helpful to prevent individuals from eating unhealthy foods (4). An unhealthy lifestyle that includes poor eating habits and unhealthy diet choices can lead to various chronic diseases including obesity, diabetes, dyslipidemia, hypertension, and cardiovascular diseases (CVDs) (5, 6). For example, obesity has become a prime cause of morbidity and mortality in many developed countries being a risk factor for several diseases (7). In the context of the prevalence of obesity among all age groups, it has become imperative to understand the satiating potential of foods as the energy intake of humans can be accurately predicted based on appetite sensations (8).

Food intake is regulated by different factors including organoleptic properties, environmental factors, metabolic influences, physiological factors, social influences, psychological influences and food likes and dislikes (9). In the early stages, satiety is primarily influenced by orosensory and cognitive factors as previous experiences with taste, texture, flavor, aroma, and palatability drive the urge to eat. Similarly, meal quantity affects the digestion process, while post-meal absorption is affected by the nutrient status of the gut that in turn governs satiety. The interplay of different variables governing satiety and satiation is presented in Figure 1. (10). Food intake is compulsive action as mealtime continues, inhibitory influences from a variety of sources (sensory, gastrointestinal, hormonal, neurological, and cognitive) increase, bringing the meal to a halt. Satiation being a sophisticated inhibitory process integrates all these factors and brings a meal to an end (11). Meal size is determined by consumer satisfaction and many variables contribute to the inability to eat again until the following meal after one eating session has ended. The aspects of the "Satiety Cascade" were conceived as stimulatory and inhibitory impacts. The Satiety Cascade combines sensory, cognitive, post-ingestive, and post-absorptive components to reduce the desire to eat for a certain period. Satiety and satiation are strong processes for regulating total daily energy intake and expenditure because they include the suppression of hunger (12). Two foods with the same nutritional content may have distinct appetite-stimulating effects. This is because food consumption, aside from the metabolic effects of nutrients in the gastrointestinal system, contributes to the process of controlling appetite. The satiety cascade describes the signals that impact the primary appetite-control behaviors, such as food selection, satiation (the amount of food ingested within a meal), and satiety (the extent to which hunger and eating are suppressed between meals). The satiety cascade model predicts that the main drivers of satiation are early pre-ingestive signals from cognitive and sensory processes, and that cognitive, sensory, post-ingestive, and post-absorptive signals are combined to determine the experience of satiety, highlighting the integrative nature of satiety (13).

Although internal signaling systems (for the drive and suppression of eating) stimulate and inhibit eating behavior to regulate the internal environment (tissue needs, energy stores), sensory and external stimulation of food intake also plays a role as a hedonistic dimension of appetite. Likewise, the type of meal, timing, frequency, palatability, portion size and psychological factors also affect appetite (13). It is crucial to take into account the impacts of both individual and food variances for holistic studies that concentrate on the study of human satiety responses to foods. Multiple domains (physiology, psychology, eating and type of food) must be taken into account in order to comprehend the factors that influence perceived satiety, and there are significant individual variations that are in part influenced by external and internal factors (14). Therefore, it is essential to analyze the physiological as well as behavioral aspects to completely understand the role of satiety and satiation in individual eating behavior. Consequently, we have focused here on the methods of satiety measurement, factors affecting satiety, and variation in satiety response among different food groups. Most of the previous reviews have individually discussed the role of body composition (15), sensory specific food cues (16), taste perceptions (17), gut microbiota (18), energy density (19), physical properties of food (20), and intestinal hormone receptor (21) modulated effects about food intake, satiety, or satiation. However, a recent appraisal of the strength of evidence for external or internal factors influencing appetite has not been reviewed. What is also not clear from the work presented so far is how critical it is to integrate different internal or external factors either food-related and personal factors controlling food intake

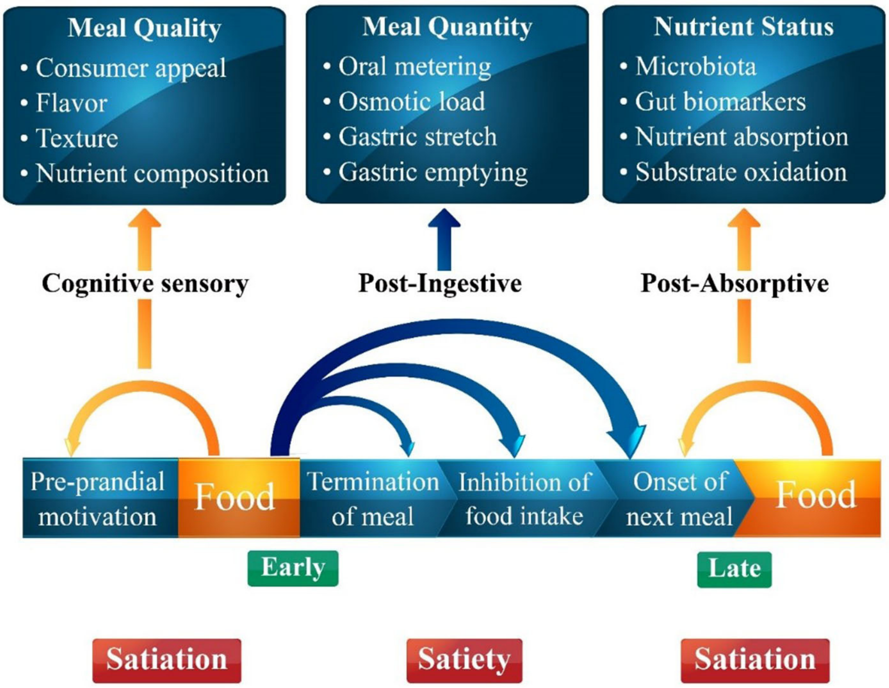

FIGURE 1
A schematic figure of the different components of the food before and after food intake determining satiation and satiety adapted from the satiety cascade model of Blundell and Gibbons.

to maximize the individual potential for improved satiation or satiety. This review aims to gather relevant existing knowledge on food intake regulation and satiety considering the role of the most promising factors involved in lowering the energy intake or controlling food intake that ultimately helps in obesity reduction or other chronic diseases. Core evidence for the satiating potential of different isocaloric foods is also carefully summarized in this study. The role of satiety hormones and modulation of different orosensory cues along with the effect of food texture expected satiety on portion sizes, age, gender, and the response of different functional foods from various food groups on ingestion in delaying the appetite sensations has been discussed. Moreover the interplay of bioactive ingredients and functional foods in relation to appetite control, satiety or body weight reduction has been considered.

Measurements of subjective satiety

Satiety is a subjective measure of appetite as people feel hunger differently. Various methods have been used for the measurement of satiety owing to the difference in standardizing the test instructions to participants (22) and the lack of standardized protocols (22). The major problem in the assessment of satiety is attributed to the non-uniform perception of satiety sensations among different individuals. Purposely, assessment of satiation and satiety is normally carried through either of the following methods: subjective appetite rating, *ad libitum* intake, and physiological measurements (23).

Previously, many other scales have been employed for the measurement of satiety such as a seven-point scale (24), a labeled magnitude scale (25), and a triangle rating scale (26). Nevertheless, VAS remains the most frequently used scale in subjective measures of satiety. Earlier, a satiety index was developed by Holt et al. (27) in Australia to compare the satiety value of different foods using a number or value. In this context, a VAS was used to assess the subjective response of the participants to different food items. The data was recorded by taking the appetite ratings before and after 120 min of food ingestion. The satiety response curves of the test foods were compared with the reference food (white bread) (27). The satiety rating of the bread was assigned a score of 100, while the satiating potential of all other foods was determined based on ranking against reference bread as illustrated below (Equation 1).

$$Satiety\ index\ (\%) = \frac{Sample\ score}{Reference\ bread\ score} \times 100 \qquad (1)$$

Subjective ratings of appetite have been conducted using a visual analog scale (VAS). The scale comprises a scale that is either 100 or 150 mm in length. The subjects in question rate their feeling of appetite by placing a mark on the scale in response to different questions posed, whereas the distance from left to the marked point is recorded to calculate the satiety value. A graph is developed by taking the post-meal consumption appetite readings after every 2–3 h interval. VAS is a reliable and valid tool for satiety measurement under controlled settings (28). The following questions form the basis of the VAS scale assessment including (1) How hungry do you feel? (2) How much food do you think you could eat? (3) How strong is your desire to eat? (4) How full do you feel? usually asked to complete the assessment (28). These measurements offer insightful data on sensations that are challenging to record using other techniques. Pen and paper were used to administer VAS in the past since it was quick and simple. However, as each line must be physically measured and entered into a database one at a time, the pen-and-paper technique of data gathering is frequently time-consuming and subject to human error. Portable handheld computers have been created to electronically administer appetite scales, solving the issues with pen and paper (Electronic Appetite Ratings System or EARS). The laboratory test meal approach has been used in some significant experimental investigations to support the validity and reliability of VAS as a measure of the intensity of the incentive to eat (29).

The relationship between energy and the satiety score of different foods can be a useful addition to nutrition facts tables on the food labels. Similarly, a satiety quotient (SQ) describes the satiating efficiency of foods and the amount of energy consumed. The SQ was computed by dividing the difference between pre- and post-eating episode assessments of motivation to eat (pre minus post) by the energy content intake during the episode of eating. Subsequently, the SQ can be calculated using the following expression (30) (Equation 2).

$$\text{Satiety Quotient} = \frac{\text{Pre} - \text{eating episode rating (mm)} - \text{Post eating episode rating (mm)}}{\text{The energy content of the test meal (kcal.)}} \quad (2)$$

However, since other factors affect fullness and satiation, subjective sensations do not give a complete picture of appetite control and calorie intake. This method also enables the calculation of the satiety quotient about the energy/weight content of the meal offered, allowing for the measurement of subjective appetite about the quantity of energy consumed. However, the results of such studies may be found to be more meaningful when the eating pattern and study schedule resemble in terms of eating duration (3–4 h) that is followed normally by participants (29). Following the start of preload ingestion, a typical *ad libitum* test meal made up of well-known, easily accessible foods and water are usually provided. Then subjects allowed for a specific window of time to consume till they are satisfied and are allowed to ask for more food if desired. The idea behind the method is that interventions that increase satiety will cause people to consume less during a typical meal and vice versa (22). Since hunger is a definite factor in determining food intake, the participants must be in similar appetite states while evaluating energy intake. Before being served an *ad libitum* meal, participants' access to food and beverages should be restricted to maintain a consistent level of hunger among participants and across situations (29).

Integrating physiological measures to record changes in satiety indicators in the postmeal interval can improve sensitivity and discrimination in satiety responses to various treatments. Postmeal phenomena such as changes in gastric emptying rate, circulating levels of certain gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), cholecystokinin (CCK), and polypeptide-P (PP), and suppression of ghrelin are more pertinent when exploring satiety (22, 29). Studies on these physiological biomarkers provide evidence of their role in regulating appetite and calorie intake. The practicality of quantifying these peptides has several challenges. Since the peptides break down so quickly, it is necessary to implement regular processes to stop this. Thus, these postprandial investigations detecting physiological indicators associated with appetite are very challenging and expensive to conduct.

Like other electronic tools, near-infrared (NIR) spectroscopy, a potent optical analytical technique, is effective for the non-destructive and label-free evaluation of biochemical, molecular, and structural information in biological tissues, including human tissues. Human tissue has biomarkers that reveal information about metabolic health and body composition, such as the proportion of lean to fat muscle tissue and body fat. A hand-held portable NIR equipment was tested by Ni et al. (31) for its capacity to capture the spectra of human tissues (arm, face, ear, mouth, and wrist) and to predict satiation, fullness, and food intake in participants from a sensory investigation. Results suggested that it would be able to evaluate the complicated interactions between humans and food by using the NIR spectra of tissues as a proxy. Variations in the cross-validation statistics were also noted, and they were strongly influenced by the type of tissue examined, metabolism, and body composition. A variety of electronic devices have been approved for use in assessing appetite for hunger or fullness as recent models made possible by their economic and other practical advantages.

A variety of factors might affect the feeling of fullness. So for a true assessment of meal termination, only one component should be permitted to change at once. It is rather unclear how similar studies of a kind may exist because study designs can vary greatly. Comparisons can be performed if the study is planned to take into account the aforementioned factors, although caution should be used when approaches diverge significantly. Appetite measurements should include a comprehensive collection of measurement techniques that allow for the evaluation of the potency of the motivation to eat, key food choices, and hedonic processes that modify the homeostatic system (29).

Factors affecting satiety

The influence of different internal factors (Figure 2) and external factors (Figure 3) on appetite, satiety and satiation have been discussed that possibly affect food intake. Although the literature on these external or internal variables and satiety is complicated yet all these factors have been potentially studied with eating behaviors.

Personal factors

Physiological

The gut is the largest organ of the body for hormone production as well as the presence of various enterocytes (32). Initially, satiation is influenced by the stomach distension as mechanoreceptors send signals to the hypothalamus *via* the vagus nerve located on gastric distension. When food passes through the gut, multiple peptides are released from the specific enterocytes of the stomach and small intestine including cholecystokinin (CCK), neurotensin, gastrin, glucagon, somatostatin, peptide YY (PYY), bombesin, and glucagon-like peptide-1 (GLP-1) (33). Hence, the physiology of food intake regulation involves precise coordination between neuronal and hormonal signals. Among them, ghrelin (orexigenic hormone) is the only hormone released from the oxyntic glands of the stomach which triggers appetite and favors feeding by enhancing the incentive and hedonic response to food-related cues (34). Other hormonal signals released from either of the upper or lower intestinal tracts involve leptin, PYY, CCK, and GLP-1 are responsible to suppress hunger (29, 35). The GLP-1 (anorexigenic hormone) releases from the small intestine in response to contact of glucose with L-cells, causing a drop in hunger. Thus, slow digestion of food can sustain prolonged intestinal contact with glucose, thereby improving satiety. Likewise, CCK releases from the small intestine in response to the fat and protein contents of the food being ingested to suppress the appetite as soon as the signal reaches the nucleus solitarius tractus (central nervous system) through the vagus nerve (36). The long-term food intake is regulated by the leptin secreted from adipose tissues, thereby maintaining energy balance (33).

Gut microbiota

The interplay between gut microbiota, satiety hormones and energy intake along with underlying mechanisms have been well studied. Often, obese people tend to be insulin resistant, and modifications in host bacterial interactions with dietary intake can be beneficial in suppressing the appetite (37). Enteroendocrine cells generate intestinal hormones such as CCK, GLP-1, and PYY, which play an important function as signaling systems. The stomach and various brain areas have been found to contain receptors for these hormones, emphasizing the gut-brain relationship in satiation mechanisms. Diet can modulate the intestinal microbiota, which interacts with enteroendocrine cells, by delivering certain nutrients that cause changes in the gut ecology (dysbiosis) due to hyperphagia. As a result, macronutrients may activate the microbiota-gut-brain axis *via* mechanisms such as particular nutrient-sensing receptors in enteroendocrine cells that cause hormone release. This results in a reduction in appetite or an increase in energy expenditure (38). In this regard, prebiotics has demonstrated their efficiency by increasing the expression of anorexigenic hormone (GLP-1) which in turn acts on the brain to signal hunger or satiety. The proposed mechanism is considered to reduce the gastrointestinal transit time by acting as an ileal break (39). Likewise, the production of short-chain fatty acids (SCFA) by the gut bacteria while metabolizing non-digestible carbohydrates has been shown to upregulate gene expression of proglucagon, the precursor to GLP-1 and PYY43 in the intestinal tract (39). This phenomenon leads to increased satiety and decreased food intake after the meal. Short-chain fatty acids can trigger intestinal gluconeogenesis through a cyclic adenosine monophosphate-dependent mechanism (40) which has positive effects on glucose and energy balance. Propionate, for example, is an energy source for epithelial cells, but it is also transported to the liver, where it contributes to gluconeogenesis. Because of its interaction with gut receptors, it's becoming more well recognized as a key component in satiety signaling (41). Likewise, ghrelin levels have shown negative correlations with *Lactobacillus*, *Bifidobacterium*, *Blautia coccoides*, and *Eubacterium rectale*, whereas the inverse was observed with *Prevotella* and *Bacteroides* (42). This signifies the role of gut microbiota in the satiety regulation and interaction with ghrelin and leptin. Moreover, butyrate production in animal models has been associated with serotonin levels which are an important neurotransmitter in the brain and gut, involved in the regulation of satiety and body weight (42). It is not clear, if gut microbiome composition changes are driven by a decrease in leptin action, as a consequence of hyperphagia, physiological modifications associated with obesity, or other leptin actions independent of food intake and adiposity. Moreover, the leptin signaling pathway related to leptin receptor (LEPR) extracellular domain mutation suggests its role against gut pathogens and it seems that leptin signaling may also have a role in modulating gut bacterial microflora, independently of food intake, by regulating gut antimicrobial peptides expression (43–45). Moreover, gut microbiota might be associated with leptin resistance, which is in general developed in obesity, throughout interfering hypothalamic and brainstem neural processes, involved in

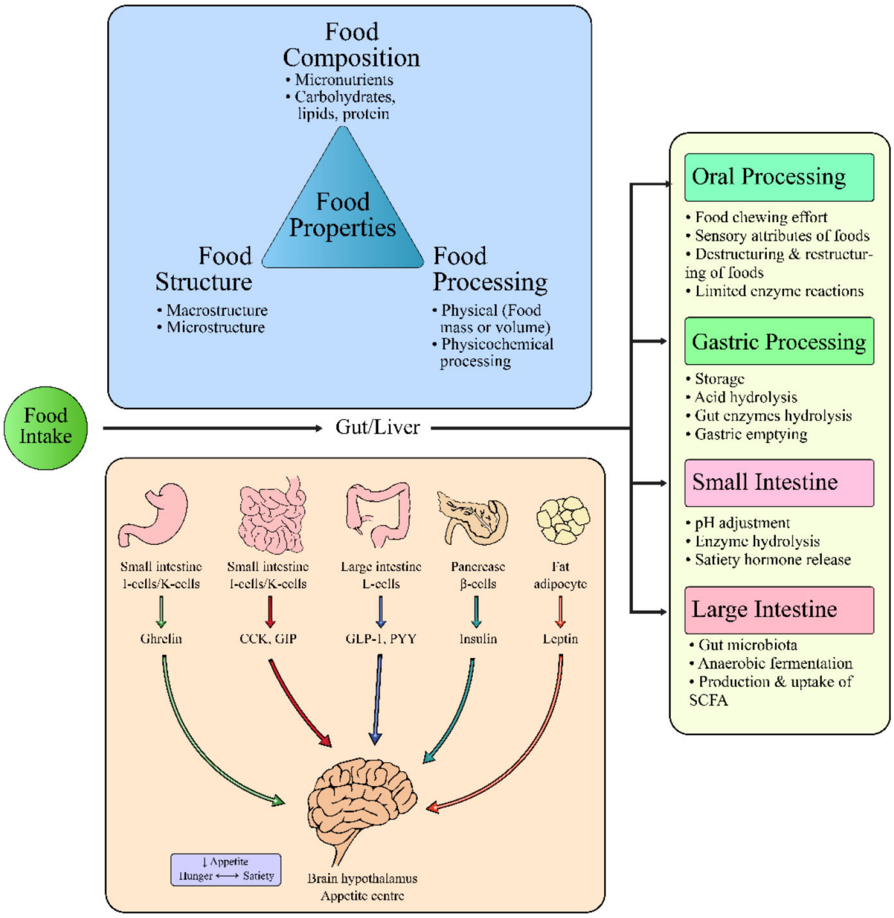

FIGURE 2
Interrelation of food properties and internal factors controlling food intake, satiation or satiety.

feeding and energy balance control (46). All of these suggest that gut microbiota modulation could be a novel therapeutic target in obesity focusing on leptin signaling (47). Besides, the prebiotic effect related to gut microbiota modulation refers to a higher leptin sensitivity and glucose tolerance, and lower oxidative stress and inflammation (48).

Although clinical trials have shown alteration in human gut microbiota after consumption of maize, whole grain wheat, and barley, there is no functional link between fullness and gut microbiota due to a lack of valid satiety assessments (18). Decreased sensation of hunger on *ad libitum* lunch intake in healthy young men by consuming wholegrain rye may be partly mediated by colonic fermentation as *in vitro* fermentation profile of rye kernel also confirmed SCFA production after 24-h of fermentation study (49). Likewise, when compared to the breakfast of refined wheat bread, rye kernels improved satiety most substantially, both immediately and in the face of a second meal, as evidenced by lower energy intake at lunch and self-reported VAS ratings. The researchers hypothesized that greater microbial fermentation or increased fermentation metabolites could be observed, as seen by higher breath hydrogen levels after eating whole grain rye bread than refined grain wheat bread. These fermentation products may help with glucose management and satiety by delaying the release of ghrelin, the hunger hormone (50).

Sociocultural

Mostly, physiological aspects of appetite regulation are studied, however, it is imperative to include social dimensions of satiety for a better understanding of the underlying phenomenon. Variations in specific cultural patterns of cuisines and food intake affect satiation and satiety and are primarily dependent on meal size. Furthermore, consuming food with other persons may increase the intake by up to 44% and it tends to increase successively in the presence of more people. Therefore, the company of the eaters such as family, spouses, friends, and colleagues at mealtime influences the energy intake. Moreover, eating foods under different conditions and the nature of the companions

FIGURE 3
Overall external factors affecting food intake, satiation and satiety.

control the energy intake, as obese individuals tend to consume more food in the presence of obese in contrast to non-obese individuals (51, 52). Social isolation, poverty, and loneliness are the other predominant factors regulating food intake, thereby in turn appetite sensations. Other determinants of food choice include socio-economic factors, media literacy level, social inequality, family dimension, health, ease of access, occupation, taste, food preferences, knowledge, peers, friends, parental education; nutritional quality of food, cooking skills, life course, past experiences, ethnic customs as well as past eating habits (53). Physical conditioning and emotional reactions to the social setting in which eating occurs can also affect how you feel about being full. Parents substantially shape the context in which children encounter food by regulating, encouraging, restricting, and rewarding food (54). Although our determinants for food choices are greatly influenced by biology. Though the biological factors that regulate food intake can be modified by disease conditions, experience, or learning. Other social and environmental influences also affect the relationships between the person and their dietary choices relate to familiarity and learnt safety, conditioned food preferences, and conditioned satiety (55).

Psychological

Satiety is a complex phenomenon and must be interpreted from both metabolic and behavioral perspectives. The psychobiological dimensions of satiety involve three events i.e., hunger perception, food cravings, and hedonic sensations. Consumption of food triggers various physiological events that in turn control the neurochemical activity of the brain which represents the desire to eat and willingness to refrain from eating (56). Psychological aspects that govern meal-by-meal appetite make it necessary to highlight their impact as a conditioning factor of satiety regulation.

Individuals on a weight-loss dietary regimen have demonstrated that appetite is merely linked with distinct psychological phenomena such as feelings of deprivation, increased reinforcing value of food, cravings, increased subjective appeal of energy-dense foods as well as an increased central nervous system (CNS) reward system feedback to calorie-rich foods. Regulation of food intake by maintaining homeostasis between reward and inhibitory controls of food cues plays an important role in conditional eating and subsequent appetite responses (57). Considering sensory-specific satiety, concepts of food acceptance and rejection play an important role in determining personal eating patterns. Likewise, cognition affects the eating process as conditioning to specific food cues can alter the food intake pattern. Learning about different foods and developing likes and dislikes throughout life are associated with certain conditioned and unconditioned reflexes, affecting behavioral eating patterns. Similarly, foods consumed before exercise can improve cognitive functioning and positively influence the mood of people with improved appetite control (58) as a result of improved insulin sensitivity and glucose response after a meal.

Environmental factors

Although overconsumption norms are prevalent in our society, studies suggest that portion size directly impacts food intake regardless of hunger level and taste preferences. Along with different consumption patterns and utensil size illusions, environmental interferences such as watching television (T.V.) or listening to music can affect both food selection and intake. Besides these dynamics, watching TV during eating is the most important factor affecting satiation and satiety as it directly influences energy intake. Reportedly, viewing TV can significantly impact appetite ratings with an increased food or energy intake (59). Likewise, a study was designed to reveal the outcomes of watching TV while eating using two energy-dense foods. Participants were randomized into two groups receiving macaroni and cheese or pizza as a test meal. While watching TV programs of their choice, readings for energy intake, hunger, satiety, and palatability were taken. Results revealed 71% higher energy consumption from macaroni and cheese and 36% from pizza (60). Similarly, another study concluded that consuming a meal while watching TV not only enhanced energy intake at mealtime but also affected the normal mealtime satiation or satiety followed by reduced satiety signals from previously consumed foods (61). People usually eat those foods with enjoyment that they like in contrast to the less-liked ones, as they experience more satisfaction, pleasure, and satiety after consuming the meals of their liking (62). Environmental factors that may influence food intake and food selection include the size of the portion, the presence of other people, the location and the time of consumption. More specifically, it has been demonstrated that the color of the plate ware, the packaging, and the atmosphere all have an impact on food consumption (63). Consumers may be able to prevent overconsumption by being aware of environmental cues such as illusions, distractions, portion sizes, and variety. As appetite reflects the expression of the urge to eat and the behavior that is directed toward the intake of food and drink items readily available in the environment. Therefore, environmental or contextual factors that may be implicated in meal termination should also be taken into consideration (29).

Gender differences

Gender difference affects food intake regulation, appetite control, and management of healthy body weight. Females are easily satiated during eating as compared to males due to the involvement of certain hormonal and neuronal activation (64). The impact of gender difference on hunger scores revealed that women ate the given amount of isocaloric *ad libitum* food satisfactorily while men did not satiate easily and consumed significantly more (65). The difference in body composition of males and females is an important contributor to the variable food/energy intake. It is pertinent to mention that women possess significantly more body fat when compared to men, hence having more leptin levels in the body (66). The leptin is secreted from adipose tissues and promotes satiety by acting on the hypothalamus. Owing to higher adiposity in females, leptin secretion is relatively more as compared to males, which results in declined food intake and energy expenditures (67). When the fat cells increase in number, leptin levels increase proportionally, and then bind to LEPR in the brain, which sends signals to inhibit food intake and increase energy expenditure. No matter how, when caloric intake exceeds energy expenditure (positive energy balance) is sustained for critical periods, weight gain occurs (68). The majority of obese people have hyperleptinemia and do not respond to leptin therapy, showing leptin resistance and casting doubt on leptin's function as a human energy balance regulator. Chrysafi et al. (69) showed that long-term leptin treatment lowers fat mass and body weight and transiently modifies circulating free fatty acids in lean slightly hyperleptinemia people, but short-term leptin administration alters food intake during refeeding after fasting.

Age differences

Age is an important element that regulates satiating efficiency of foods since sensory-specific satiety declines in old age due to age-associated changes (increases in intensity discrimination) for taste and smells, reducing energy intake. Therefore, old-aged people can easily get satiated and become leaner with increasing age (70). In this perspective, a study was conducted to assess the age mediated difference in sensory-specific satiety. For this purpose, adolescents, young, older adults, and elderly persons were recruited for the study. The results obtained showed distinct differences in sensory-specific satiety among adolescents and the elderly. This explains the limited food choices in elderly persons owing to a decrease in food pleasantness which may lead to serious health threats (71).

Effect of chewing

The impact of chewing on satiety is evident from the fact that people usually chew less if food is more palatable, hence promoting food intake. Thus, chewing food can alter the eating rate and digestion. The chewing rate is associated with satiety and is usually higher for mixed meals when compared to single food (72). The mastication of almonds resulted in a decline in GLP-1 (orexigenic hormone) and an increased fullness after 40 chews as against 25 chews (73). Chewing may enhance or reduce hunger and relative food intake through gut hormone response modification. For example, chewing gum has enhanced the feeling of satiety in obese as well as healthy weight women (74). Moreover, video recordings of chewing gum at different frequencies also showed variations in chewing (75). The findings of the study revealed that the rate of chewing particularly depends on the type of food being consumed. Increased rate of chewing in each mouthful for sustained meal duration had shown a decline in food intake (76). Similarly, a decreased snack intake was observed after 2 h of having lunch with prolonged chewing. Thus, a higher number of chews promote slow eating and may help obese people with less caloric intake (77). Furthermore, the rate of food biting has been associated with energy intake, with a slower rate being more useful to decrease food intake. Interestingly, obese people usually take large bites as compared to lean individuals, affecting the rate of food ingestion (swallowing) and successive increase in energy (78). In fact, recent research showed that extending the time between chewing and swallowing reduces food consumption and boosts fullness. Although it has been demonstrated that delaying eating can prevent weight gain in children and adolescents, it is unclear if slowing eating by increasing the number of masticatory cycles or lowering the mastication rate is a practical way to support weight management (79).

Physical activity

Physical activity is another important parameter governing appetite by improving the sensitivity to physiological responses regulating satiety. Purposely, the effect of physical activity on appetite along with satiety scores was assessed in obese women (average BMI of ?37) after 20 min of brisk walking. The results suggested that even moderate physical activity is vital in modulating the role of postprandial peptides (insulin and leptin) in the short-term regulation of food intake (80). Therefore, consistent physical activity can improve appetite control by improving satiety signaling. However, specific actions, intensity, and duration of exercise can affect physiological elements of satiety, which also varies from person to person based on individual physiology (81). Hence, regular exercise has a strong potential to control appetite and satiety (82). In general, leptin is overexpressed in obese individuals, and its altered expression leads to leptin resistance, which implies mechanisms interfering with leptin's ability to reach targeted cells due to decreased LEPR expression or altered signaling. Genetic variations in the LEP gene can modulate its circulating levels and interfere with various pathophysiological processes (47). In this context, in the last few years, increased obesity prevalence as a consequence of a sedentary lifestyle and low physical activity has been linked to systemic, chronic low-grade inflammation processes through adipocyte-secreted hormones (adiponectin, leptin, resistin, and ghrelin), growth factors and proinflammatory cytokines (83). Studies on rodents and humans provide evidence that the majority of exercise induced favorable effects on obesity are linked to lower leptin levels and improve leptin resistance. For instance, in obese adolescent girls, a 12-week combined resistance and aerobic exercise training efficiently reduced body weight, waist circumference, and serum leptin levels, hence reducing central leptin resistance (84). Combining resistance and aerobic exercise training also improved the cardiometabolic indicators of older men with obesity along with a reduction in leptin levels (85). High-intensity interval training, other than combined training, also decreased body fat and inflammation in obese postmenopausal women, along with a significant drop in leptin levels (86).

Sleep and circadian rhythms

Sleep is also another important factor for appetite control and laboratory studies demonstrated that sleep deprivation impairs insulin sensitivity and glucose disposal throughout the body. Individuals recruited in a study trial stated that sleep restriction lowered participant-perceived fullness or satiety as well as suppressed the postprandial lipemic response and decreased satiety (87). As the SQ governs the extent to which a meal can minimize subjective appetite sensations, SQ in response to a standardized meal was assessed in overweight or obese men according to sleep duration for a later bedtime and poor sleep quality in association with energy intake. Results revealed that short-duration sleepers had a lower mean SQ than recommended sleep duration sleepers without impacting overall energy intake (88). Interestingly, another study found greater total food-craving scores in subjects in association with increased daytime sleepiness, when participants were assessed by a 7-day sleep-hunger-satiety diary (89). A lot of people who live at home don't get enough sleep. When volunteers were experimentally sleep-restricted but had unlimited access to food, they consumed more calories than when they are not sleep-restricted; these calorie increases are often observed in post-dinner snack patterns (90, 91).

Energy consumption during inappropriate circadian periods is one potential reason for negative health effects during circadian disturbance and inadequate sleep. Lab investigations have shown that when people are given meals during the circadian night (when melatonin levels are high), they have a lower energy response than when they are given meals during the day along with impaired glucose tolerance (92, 93). There is compelling evidence that energy consumption later in the day may contribute to ill health during both circadian disturbance and inadequate sleep. Though circadian rhythm was not altered in conditions with sleep episodes lasting <6 h per night (i.e., chronic sleep restriction) in which participants were given a diet designed to meet caloric needs (94). This showed that circadian timing, rather than sleep limitation, might play a significant role in hunger patterns. However, it is uncertain how several days of energy consumption during the circadian evening and night, as well as at a period when melatonin concentrations increase favoring sleep (i.e., chronic circadian disruption) may affect hunger, appetite, and food choices (95).

The integration of internal circadian rhythms and external cues such as the light-dark cycle and dietary composition is critical for survival and requires temporal partitioning of daily food intake. These internal and extrinsic variables are interrelated, with circadian rhythm misalignment encouraging body weight increase and calorie-dense diet intake increasing the risk of obesity and blunting circadian rhythms (96).

Genetics

Mealtime, the quantity of food consumed, and food preference are all influenced by a complex interaction of physiological, psychological, and social interactions along with genetic variables (97). Heritability and linkage analysis of individual food-consuming behavior measured by the three-factor questionnaire (TFQ) provides evidence that eating behavior traits are heritable. A growing body of evidence links hedonic signaling to the obesity epidemic in addition to the role of the hypothalamus and hindbrain in homeostatic food intake and satiety. The hippocampus is particularly rich in genes associated with human genome-wide association study (GWAS) obesity loci. A high-fat diet and obesity have frequently been associated with hippocampal atrophy, which may potentially affect responses to taste. The hippocampus may help regulate meal size (98, 99). The nucleus accumbens has been studied for obesity therapy because it can impact food intake pathways (100). In the insula and substantia nigra, areas implicated in addiction, motivation, and reward-seeking behavior, a recent study found substantial gene expression enrichment of top obesity/BMI-associated loci (101). Depending upon the nutritional status for eliciting the act of producing a satiety response, the hypothalamus communicates with the insula (102).

The fat mass and obesity-associated gene (103) is one of the most important obesity-associated genes discovered using GWAS. In the first intron of the FTO gene, several variations have been discovered that are linked to increased calorie ingesting, body fat, weight, and other adiposity measurements (104). About a 1.7-fold increase in obesity risk has been observed in patients that are homozygous for the "A" allele relative to the low-risk "T" allele owing to one of the best-studied FTO rs9939609 variants (105). Besides, postprandial appetite reduction in subjects noted that are homozygous for the A allele because of dysregulated circulating levels of acyl-ghrelin, suggesting that variations in FTO may change the action of ghrelin, the hunger-promoting gut hormone (e.g., reduced satiety response) (106).

The satiety pathway is usually well controlled. The LEPR and the melanocortin-4-receptor (MC4R) genes are two of the most investigated genes expressed in the brain, revealing biological mechanisms not yet fully elucidated. Several single nucleotide variations in LEPR have been linked to severe obesity, including Lys109Arg and Gln223Arg. According to recent studies, roughly 7% of the general population as well as obese persons accounting for more than 10% of the population had a coding variation in MC4R. About 20% of single-nucleotide variants in the MC4R gene have been projected to be pathogenic or likely pathogenic, emphasizing MC4R's prevalence in monogenic obesity (107).

In leptin-deficient people, leptin replacement can enhance satiety and help them lose weight. Leptin stimulates the production of a melanocyte-stimulating hormone (a-MSH), which induces satiety. Eating habit has also been connected to GAD (glutamic acid decarboxylase), which converts glutamate to GABA (g-aminobutyric acid), a brain inhibitory neurotransmitter. Disinhibition and disordered food consumption, notably higher carbohydrate intake in women, have been linked to two specific GAD variations, rs7908975 and rs992990 (97). Even though in the last years, several complex mechanisms related to energy regulation and obesity have been proposed, further studies are needed for a better understanding of interactions between genetic, environmental, and lifestyle factors that contribute to obesity (108).

Mood and food cravings

Food desire is considered to be one of the main elements influencing eating behavior, along with hunger, which is brought on by food deprivation or fasting. Although healthy adults with typical eating habits experience food cravings, research indicates that intense food cravings may be a risk factor for binge eating, which may lead to weight gain and obesity. Food cravings are viewed as a motivational state that is conditioned in response to sensory, environmental, or interceptive inputs (109). Moreover, Reents et al. (110) used a food cue-reactivity paradigm on normal-weight females to more thoroughly investigate these impacts on momentary food seeking. The states of food deprivation (hunger vs. fullness) and mood (negative vs. neutral) were changed systematically. In comparison to stated states, the self-rated craving was much higher when one felt hungry. Additionally, high-calorie foods reduced cravings in a neutral mood; hence, people who were hungry or satisfied preferred savory food and sweet food, respectively. This distinction between the effects of savory and sweet foods was not seen in a depressive mood. In conclusion, hunger has a significant impact on food cravings, which are further influenced by emotional state.

Gut enzymes and gastric emptying

Enzymes can greatly contribute to digestion-induced changes in the food structure. Since gastric cells secrete hydrochloric acid in reaction to food entering the stomach, the stomach has a strongly acidic environment with a pH of roughly two. The stomach secretes two enzymes that help break down proteins (pepsin) and lipids (gastric lipase). Depending on the rate of mixing and acid production, salivary amylase probably continues to work on carbs in the stomach for some time (111). One of the key factors in food disintegration in the stomach is the hydrolysis of proteins by pepsin. Several food particle-specific characteristics, including the solids content, density, internal tortuosity, surface-to-volume ratio, and porosity, affect the diffusion of pepsin into food particles (112). Different fluorescein isothiocyanate pepsin diffusion coefficients of two egg white gels were reported with the same protein concentration (10 wt%) as structures induced at pH 5 or 9 were found to vary more. The pH 5 gel displayed a greater diffusion coefficient than the pH 9 gel due to the pH 5 gel's more loose, spatially heterogeneous protein matrix and homogeneous microstructure (113). To produce free fatty acids and 1,2-diacylglycerols, gastric lipases preferentially hydrolyze the sn-three position of triacylglycerols. Triacylglycerols that have been consumed by healthy persons undergo 10 to 30% lipolysis during stomach passage (112). Food macromolecules are broken down in the small intestine, which functions as an enzyme bioreactor, by the hydrolytic processes of the carbohydrates, proteases, peptidases and lipases. Low molecular weight hydrolysis products diffuse out and are then absorbed into the bloodstream. Additionally, bile acids are released from the gall bladder duct and assist to emulsify lipids which facilitates breakdown by pancreatic lipase (111). The protein conformation, the presence of cross-linkages between protein chains, binding metals or polyphenols, the particle size, and the presence of anti-nutritional factors like trypsin and chymotrypsin inhibitors also have an impact on how food proteins are digested. Additionally, inter-individual variability is important and can be influenced by factors including age, health, and the usage of common medicines like antacids. Protease inhibitors, polyphenols, saponins, phytic acid, and the presence of complex carbohydrates that prevent enzymes from accessing the protein all have an impact on how digestible plant proteins are at this level (114).

Gastric emptying has been the subject of considerable study because it is believed to have several effects related to satiety. Even while eating a small meal can cause the stomach to fill up rapidly, the stomach's release of digesta takes time. Particles larger than 1–2 mm are typically maintained in the stomach until late in the emptying stage due to the sieving effect. Usually, the release of gastric contents happens over a few min to up to 6 h or more, with the primary peak of release occurring after 1.5 to 2 h. Overweight people are said to have faster rates of stomach emptying (111). The relationship between enhanced subjective satiety signals in humans and a decreased stomach-emptying rate, or prolonged gastric residence time, has been established. It has been demonstrated that diets with the same macronutrient composition, whether they are solid or liquid, affect stomach emptying and the release of satiety hormones in the intestines differently. For example, in a study using liquid and gelled lipid-protein emulsions, it was discovered that the liquid diet caused a faster release of nutrients into the lumen, leading to a more rapid nutrient sensing at the proximal part of the small intestine because higher levels of the gastric inhibitory polypeptide (GIP) were discovered in the plasma of liquid-diet-fed rats (114).

This is because food deconstruction and rearrangement during gastric passage have a huge impact on how nutrients are absorbed later on and how full you feel. Food disintegration, viscosity changes, nutrient redistribution, and gelation are all effects of intragastric food structure that can affect gastric distention and emptiness and consequently, satiation and satiety (112).

Food-related elements

Sensory attributes

The sensory characteristics of food play a vital role in the regulation of food intake. Mostly, the appearance of food influences eating, which resultantly governs the amount of food to be consumed. Previously, the potential impact of sensory attributes of food including its appearance, odor, taste, and texture on satiety and satiation has been documented (16). Food odors have been found to either increase or decrease food intake, especially based on individual perceptions. Individual preferences for different odors mainly affect the palatability of foods (115). The palatability of food thus affects the eating process to a great extent as positive hedonic signals before meal initiation can enhance food consumption (16). The impact of food labels indicating a food's satiating attributes has also received less attention. Since expected satiation has been demonstrated to affect hunger ratings and food intake, such labeling may have an impact on how much food is consumed. As this mechanism may be involved in the impact of satiation labels on intake, Hendriks-Hartensveld et al. (116) observed that the effects of such labeling on the magnitude of sensory-specific satiety are a relative decline in the pleasantness of food during consumption experienced after eating the meal.

Food structure

Understanding the role of food structure in satiation and satiety becomes tougher as hunger and fullness are influenced by physiological, psychological, and other physical factors before, during, and after the consumption of food. However, evidence suggests that the texture of food is an important element in the arousal of hunger sensations as it directly or indirectly influences oral processing factors such as mastication, chewing efficiency, orosensory time, and self-textural perceptions. Therefore, the texture of food not only determines the overall acceptability of a meal but also influences the satiating potential to a certain degree. The involvement of certain neurons in assessing the orosensory cues may trigger the varying palatability responses for different meals since mouthfeel differs corresponding to the texture of the food which in turn affects satiation and satiety (17).

The physical and rheological properties of foods (solid or liquid) are thought to have an influence on energy consumption owing to their perceived satiating effect (117). The impact of food consistency i.e., raw, semisolid, fluid, or pureed on satiety has already been investigated (118). The first systematic review and meta-analyses on the influences of food texture (form, viscosity, structural complexity) on satiety were presented by Stribitcaia et al. (20). Results delineated that as compared to liquid and low viscous food, both solid and more viscous food reduce hunger. It was also observed that there was an association between viscosity and fullness as well as a moderate relationship between food form and food consumption was also noted. Highly viscous liquids provide more satiety as compared to less viscous liquids. This phenomenon might be explained by the decreased eating rate since a spoon or straw is required coupled with increased engagement of muscle and tongue. As a result, the oral processing time of food is increased, affecting the psychological and physiological signals that control satiety (119).

Food macrostructure usually affects gastric retention, rate of gastric emptying, and nutrient absorption. Purposely, a study was carried out on 10 healthy volunteers to examine the impact of gastric retention on appetite sensations using isocaloric test meals. The results revealed increased gastric retention and a decreased appetite for a semi-solid meal as compared to a liquid intake. This in turn translated into differences in blood glucose and insulin responses that affect satiation and satiety. The increased viscosity in the stomach and improved sensation of intestinal nutrients leads to good appetite control (120). Moreover, the potential of food microstructure in altering satiation response may be elucidated during digestion which is strongly affected by variable particle sizes of the meal. Likewise, the effect of oil droplet size while consuming 2 mm or 50 mm in an emulsion preload suggested that not only perceived liking for creaminess affect appetite but smaller droplet sizes resulted in decreased food intake at subsequent lunch (121). In addition, compared to the milled rye kernel porridge breakfast, satiety was increased, and appetite was suppressed in the afternoon following the ingestion of the rye kernel breakfast. This influence may be attributed to structural variations alone, as the nutritional quality of both commodities was similar, including the content and structure of dietary fibers (122).

Processing also influenced the food structure and often increased the digestibility of foods when compared to raw foods. Resultantly, processing improves glucose availability and is more likely to affect satiation than satiety (123). It is pertinent to mention that unprocessed or raw foods render satiety due to prolonged gastric transit time. The findings of the study explicated that whole apple particularly reduced energy intake from the test meal. Similarly, the effect of instant oatmeal and ready-to-eat oatmeal breakfast on satiety was investigated, the energy intake was particularly reduced after consumption of instant oatmeal in contrast to ready-to-eat oatmeal cereal (124).

Portion sizes

Portion size is an important consideration in designing a healthy menu for obese patients. Usually, obese individuals tend to eat more food when offered in large portions size. Many social and cultural norms also promote larger portion sizes that in turn lead to overeating and obesity. Perceived satiation and satiety relative to portion sizes depend upon the volume of the foods (125). The effect of iso-caloric portions of seven different types of bread varying in nutrient composition and physical appearance was assessed for the feeling of fullness scores. Satiety index scores for regular white bread were found to be the lowest, without revealing any correlation between satiety and glycemic response. Besides, less energy intake at test meals was found to be associated with the participant's perceived satisfaction with larger portion sizes. There is a strong link between portion sizes and expected satiety as individual liking serves as a constant stimulus to drive the satiation and satiety sensations (126).

Portion sizes of several convenience food items have tended to gradually increase. The trend has now become common in various settings including supermarkets, restaurants, and homes. This increase in portion size is one of the major causes of the current obesity epidemic. Therefore, choosing a small portion size with a relatively lower energy density is effective in weight management programs. Conversely, sustained consumption of increased portion size can particularly enhance the energy intake which leads to increased body weight (127).

The sensitivity to portion size also differs with age since children <3 years of age consume a constant amount of food irrespective of portion size as they are more sensitive to essential mechanisms of satiation or satiety. Though, when the large portion sizes were served to 5 years old children, energy intake was significantly increased due to environmental cues acquired with the growing age (128). Likewise, an up to 15% increase in energy intake was observed when 4 years old children were served with double portion sizes (129).

Currently, USDA's recommendation to control portion size and increase smart food choices includes the implementation of USDA's MyPlate. The USDA suggests filling half of your plate with fruits and vegetables, one quarter with grains (half of which should be whole), and one quarter with protein, along with a portion of low-fat or fat-free dairy (130). Calorie restriction, and portion control methods have long been used in primary care-based obesity management. The MyPlate-based obesity treatment strategy, in contrast, promotes consuming more high-satiety/high-satiation foods and does not require calorie counting (131).

Energy density

The energy density of the food plays a key role in energy consumption as satiating efficiency is largely affected by energy density (19). The energy density (kJ or kcal/g) denotes the amount of energy available in a given amount of food. The energy density is governed by the food composition since foods rich in fat are energy-dense when compared to those having a significant amount of fiber. Replacement of fat-rich foods with less energy-dense foods enriched with fiber can significantly lower energy intake (19). Food with low energy density tends to increase satiety, suppress hunger, and lessen energy intake. Hence foods with low energy density resulted in a better fullness sensation. Another work revealed that devouring a large portion size and having low energy density increased the average eating time by 33%, improved the satiety response, and displaced energy intake for the subsequent meals of the day (132).

Food macronutrients

Among major macronutrients, the protein content of the food significantly affects the satiety value when compared to fats and carbohydrates (133). Apart from proteins, soluble fiber is the other promising ingredient with a high satiating ability. Although attributing satiety to a single factor is not very meaningful, a variety of food attributes including structure, complexity, composition, etc., often act in combination at more than one level.

Carbohydrates

Carbohydrates are a diverse group of biomolecules consisting of a single (monosaccharides), two (disaccharides) few (oligosaccharides), and multiple monomers (polysaccharides). The impact of carbohydrates on satiation and satiety primarily depends upon their digestion, absorption, and metabolism, since long-chained polymers take more time for digestion when compared to sugars. Thus, changes in the level of blood glucose and satiety hormones (insulin and amylin) are attributed to a variable rate of carbohydrate metabolism. The decline in food intake after consuming carbohydrates is often associated with sensory stimulation, gastric distention, and nutrient intestinal contact (134). Hence, satiety from carbohydrates relies on the form in which it is delivered.

Considering the short-term effects of carbohydrates on satiety, individual sugars may also have a variable response, since the ingestion of glucose instantly increases the blood glucose and insulin levels in contrast to sucrose. Fructose has the least effect on blood glycemic response. Fructose also improved satiety, but the relative impact of preloads significantly controlled the food intake, since no difference in food intake was observed between 50 g fructose and 50 g glucose at 2.25 h when they were given in a mixed nutrient meal containing starch (135). Thus, the changes in blood glucose after ingestion of different sugars and subsequent decline in food intake conform well with the Glucostatic Theory presented by Mayer in 1953 which states that the onset of feeding occurs upon low blood glucose level while increased glucose level suppresses the food ingestion and governs satiation (136).

Apart from sugars, work has been conducted on the relationship between the glycemic index (GI) of foods and satiety. GI represents the increase in blood glucose in response to carbohydrate-containing foods. There is an inverse relationship between the satiety value of different foods and their GI. In this perspective, a study found that appetite and food intake were significantly suppressed on ingestion of high-GI foods as long as high blood glucose levels were persistent (137). In short-term satiety, a sudden rise in blood glucose occurs on the consumption of high GI foods, but in the case of long-term satiety, consuming low GI foods leads to a slow and steady release of glucose that helps to sustain euglycemia with improved appetite sensations. A satisfactory satiety response can be achieved using low GI diets with the same energy density. Thus, a diet with low GI and reduced energy content can be beneficial to shedding excess body weight by controlling glucose metabolism and insulin response (138).

Dietary proteins

Protein is a strong determinant of satiety as multiple investigations have validated the hypothesis that high protein diets provide an enhanced feeling of fullness. Increased protein content in the diet may result in increased thermogenesis and energy expenditure due to a strong thermic effect. Protein-rich diets elicit increased satiety as their metabolism leads to a greater number of amino acids escaping the protein synthesis channel and reaching the blood plasma, thereby serving as a satiety signal to suppress further food intake (139). Different mechanisms are involved in the satiety regulation after ingestion of protein including increased productions of satiety-related hormones i.e., PYY, glucagon-like peptide-1, and cholecystokinin coupled with a lower level of orexigenic hormone-ghrelin. It is noteworthy that not only protein-enriched diets but also isolated proteins like whey and casein have a significant effect on satiety and retain discrete satiety mechanisms (140, 141). The casein fraction of milk proteins is one such example that delays gastric emptying by getting coagulated in the acidic environment of the stomach. Unlike casein, whey proteins remain soluble at the gastric pH, rapidly passing through the stomach and resulting in faster absorption of amino acid and subsequent metabolic response. Therefore, less release of GLP-1 was observed after casein intake in contrast to whey protein, thereby promoting satiation (114).

The meta-analysis by Yang et al. (142) to compare protein-rich vs. normal protein diets has been conducted to assess postprandial satiety response. Results demonstrated that acute high protein intake (>20 % of energy from protein) did increase satiety and have a higher thermogenic effect with moderate heterogeneity between studies. Additionally, compared to normal protein test meals, high protein test meals may help control postprandial glucose. Likewise, a study was conducted to compare the effects of different proteins such as whey with or without glycomacropeptide (GMP), casein, and soy proteins. Satiety was higher after casein or soy-based high-protein meals and lower after whey-GMP-based high-protein breakfasts. Though high protein breakfast with whey and GMP satiety results due to an increase in GLP-1 (satiety hormone) (140). Another important feature of a high protein diet is an amino acid-induced increase in gluconeogenesis which may contribute to protein-induced satiety. Such an effect of a high protein diet on gluconeogenesis has been studied previously. The results revealed enhanced gluconeogenesis after the consumption of a high-protein diet. As a study carried out on appetite control drew a similar conclusion where decreased food intake was associated with high protein foods when the subjects received an isoenergetic high-protein diet (30, 0, 70% energy from protein, carbohydrate and fat) or normal-protein diet (12, 55 and 33% energy from protein/carbohydrate/fat) in a randomized cross-over design (143).

Furthermore, the comparison between animal and plant protein on satiety and glucose response in an iso-caloric breakfast revealed the usefulness of animal protein in regulating postprandial glucose response and satiety (144). Among animal proteins, eggs possess the greater potential to delay hunger as well as contain many other beneficial macros and micronutrients essential for health maintenance (145). Likewise, no difference was recorded in the satiating response of fish and beef protein (146). However, a significant decline in energy intake was observed at the subsequent meal after the consumption of fish. This decline was attributed to the slow digestion of fish, owing to specific amino acid profiles. Therefore, varying the protein sources in a mixed meal may play a significant role in metabolic kinetics. The insulin, glucose, and glucagon responses vary owing to the difference in the gastric emptying rate of various proteins (casein, gelatin, soy protein), that in a turn depends on the amino acid profiles (147).

There is consistent evidence that protein in an adequate dose has more impact on satiety as compared to corresponding amounts of carbohydrates or fat. This has also been confirmed by long-term weight loss studies, which showed that a high-protein diet was more effective in eliciting a satiety response than a low-protein diet, thus helping in promoting weight loss by reducing the amount of food intake (148). This was probably due to the greater satiety effect of protein as compared to fats and carbohydrates. However, variations in study designs cause difficulty in assessing the optimum protein dose or energy share required to detect the noticeable effects on satiety. Usually, at least 50 g of protein in each meal has been suggested to get any substantial effect on satiety, but not enough data is available to define a dose-response relationship (149).

Dietary fats

The fat-driven satiation effects are mainly induced by triacylglycerol (150) and free fatty acids. The dietary fats i.e., saturated, monounsaturated, and polyunsaturated fatty acids can be detected by the lingual lipase (upon fatty acids stimulus). Purposely, fatty acid receptors namely GPR120 and GPR40 sense the intake of dietary fat in the gut. In response to fat intake, a gut peptide released lead to altered gastrointestinal tract movement. Intestinal beta-oxidation of fatty acids is carried out through fatty acid transporter CD36, protein kinase C-zeta, protein kinase C-delta, and the 2-monoacylglycerol receptor GPR119 (151). The mechanism of appetite control and intake of fat energy vary (152). Enterocytes release a satiety signal called oleoyl ethanolamide (OEA) having an anorexigenic effect which acts on intestinal receptor PPAR alpha through vagal afferent nerves. Accordingly, the c-fos region of the brain, hypothalamic paraventricular (PVN) area, a nucleus of the solitary tract and supraoptic nuclei (SON) are activated thereby regulating food intake (153). Although a decrease in energy intake following the consumption of a high-fat diet (due to a lower amount of food eaten) has been observed, short-term studies suggest that ingestion of fat reduces not only eating time but the sensation of hunger as well, thus promoting satiation in contrast to satiety (154). Long-term studies are required to explain this increase in energy intake attributed to a variable mechanism of appetite regulation for a high-fat diet. Although some short-term studies (2–3 weeks) reported an effect of a high-fat diet on appetite suppression, however doubts were cast on their analytical approach. The high-fat diet altered the ability of the GI tract to sense fat and resulted in an enhanced energy intake. Such mechanisms have now become an important part of research to treat obesity (152).

The effect of dietary fatty acids on satiety revealed that the response of PYY was significantly lower with meals high in monounsaturated fatty acids when compared to meals enriched with polyunsaturated and saturated fatty acids (155). It is pertinent to mention that PYY is a hormone secreted by the gastrointestinal tract (GIT) to inhibit the orexigenic neuron's response to enhance satiety. Similarly, the satiation effect of medium-chain triglycerides and long-chain triglycerides is more pronounced owing to the greater post-meal oxidation of fats (156). Oxidation of fatty acids in plasma is dependent upon the concentration of glucose in the blood since insulin not only governs glucose uptake but is also involved in lipogenesis (157). Erstwhile, medium-chain triglycerides were found to be more satiating as compared to short-chain fatty acids, conjugated linoleic acid, n-3 polyunsaturated fatty acids, diacylglycerol, and small particle lipids. Such an effect was attributed to either fatty acid oxidation that enhanced ketone bodies i.e., β-hydroxybutyrate or the release of anorexigenic hormones CCK or PYY that require fatty acids with chain lengths of 12 and above to accomplish this effect (158).

Dietary fiber

The dietary fiber provides satisfaction and satiety by adding bulk and increasing the viscosity of the digesta along with GIT. The non-availability of valid biomarkers of fiber functionality related to satiety makes it difficult to compare dietary fibers for their role in satiety. Many functional fibers (inulin oligofructose, polydextrose, and resistant starch) that are not viscous have little or no effect on satiety. While other functional fibers, mostly viscous (pectin, psyllium, and guar gum) or microbiological produced (xanthan gum or pectin) increased satiety (1). Even if several studies have indicated a decrease gastric-emptying rate after viscous fibers intake i.e., pectin (159, 160), guar gum (161), β-glucan (162), and alginate (163), other opposite results showed no such effects (164–166), further research is needed for clarifying these fibers effects on energy balance and satiety, including those on the related mechanisms (167). Pectin is prebiotic with health-promoting effects, such as regulation of glucose homeostasis and lipid metabolism, and other potential health effects poorly understood until now, including obesity prevention (168). The effect of pectin is associated with improvements in insulin and glucose profiles (169, 170), and also with influences on leptin and adiponectin circulating levels, thus resulting in a decrease leptin/adiponectin ratio. Besides, the high-esterified pectin (HEP), which can be found in vegetables and fruits, is fermented more slowly in GIT in comparison with that low-esterified, the complete fermentation being carried out probably in the colon, which shows a larger and a higher variety of bacterial microflora (171, 172), thus showing a higher inhibition of glucose absorption at the intestinal level, and improved insulin resistance and of other factors related to cardiovascular health (173). HEP is a major component of soluble dietary fiber, with potentially benefic effects on metabolic disorders and obesity, showing associations with health-promoting effects related to body weight, glucose homeostasis, and lipid metabolism, even that the explanation of these benefits is not clear if it resides in the calorie intake decrement or other unveiled mechanisms (21). Moreover, HEP supplementation is able to modulate, in terms of restoring or improving, leptin/adiponectin signaling pathway and lipid metabolism throughout the oxidative/lipogenic balance in liver, being also associated with insulin and leptin sensitivity improvements, not specifically attributed to a decrease in energy intake, but to other mechanisms involved (168). Related to β-glucans, short-term and long-term studies assessed the effect of oat β-glucans in transforming diet, indicating its ghrelin, PYY, GLP-1, GIP and leptin modulating abilities (174). Besides, the oat β-glucan dietary supplementation in patients with type 2 diabetes showed effects such as improved glycemic control e.g., higher insulin secretion, but no significant differences in leptin and ghrelin, with an increase in GLP-1 and PYY that showed increased satiety perception and modified gut microbiota having healthier profile (174), contrary to other scientific reports (175). Like guar gum, xanthan results in slower gastric emptying of glucose and nutrient energy and shows resistance to bacterial breakdown, thereby its supplementation adding little, short-chain fatty acid *via* its bacterial decomposition in the gut. From the earlier reports indicating the potential of xanthan gum to be used in the dietary management of diabetes mellitus (176) and its effect on satiety in obese patients after test meal (177), recent studies conducted on the potential of using xanthan gum in emulsions. Interestingly, even if it shows lower viscoelasticity in water solution after stomach incubation, due to the reduced electrostatic repulsion in the acidic environment, thus driving to more flexible chains, on the opposite, the xanthan gum emulsion has higher viscoelasticity in the stomach based on the fat coalescence and coagulation induced by the weakness of its supporting structure (178). Whole foods consumption and their effects on satiety depend upon the kind of dietary fiber present, their viscosity as well as gut microbiota. A decrease in appetite by dietary fibers from sources like barley and oats is well reported (124). Apart from increased viscosity, β-glucan from oats imparts satiety by the increased postprandial release of cholecystokinin (124). Likewise, poor appetite ratings after consumption of wheat bran and psyllium husk had been attributed to increased viscosity and solubility of the fibers (179). Difference in insoluble and soluble dietary fiber induced satiety is subtle due to difference in action during consumption (satiation) and following consumption (satiety). In trials investigating non-viscous soluble fibers such as inulin and resistant starch, non-significant effect on satiety was witnessed. The fat content of a diet may be able to influence the total energy intake, thus, reducing dietary fats could drive to a lower total energy intake and a decreased weight gain, such statements being supported by many investigation trials. Even so, dietary fats effect on energy intake needs further assessment for clarifying if it is due to only its higher energy density or

TABLE 1 Summarizing results of studies assessing variation in satiety among food groups.

Food groups	Aim of the study	Foods tested	Satiety measurement	Results	References
Cereals	Effect of two oat-based cereals on subjective ratings of appetite	Two oat-based ready-to-eat cereals; RTEC1: Quaker Oatmeal Squares and RTEC2: honey nut cheerios	100 mm visual analog scale	Similar amounts of oat β-glucan in products but different functionality was observed as more fullness or desire to eat found after RTEC1	(189)
	Effect of rye bread breakfasts on subjective hunger and satiety	Rye bran bread, intermediate rye fraction bread, Sifted rye flour bread and wheat reference bread	100 mm visual analog scale	Significant results for rye bread in reducing appetite sensations	(190)
	Variation in satiety for cooked Philippine rice having a different glycemic index	Seven rice varieties	Satiety Quotient	Variation in satiety scores was associated with dry matter content of rice	(191)
	Effect of whole meal pasta on subjective satiety and plasma PYY concentration	Wholemeal pasta and refined wheat pasta	Visual analog scale GLP-1, ghrelin, PYY	Whole grains control the appetite instead of refined wheat pasta	(192)
	Satiety from rice-based, wheat-based and rice–pulse combination preparations	Reference bread, Semolina preparation, "Upma" broken wheat preparation, "Dalia upma", whole wheat flat bread, "Paratha" and rice flakes preparation, "Poha" Fermented rice–pulse preparation, "Idli"	100 mm visual analog scale	Fermented rice pulse combination exhibited the highest satiety scores	(193)
	Wholegrain vs. refined wheat bread and pasta. Effect on postprandial glycemia, appetite, and subsequent *ad libitum* energy intake	Refined wheat bread, wholegrain wheat bread, refined wheat pasta and wholegrain wheat pasta	100 mm visual analog scale	Whole grain wheat bread resulted in increased satiety and fullness compared to the refined wheat bread	(194)
	Effect of biscuits formulated with high-amylose maize flour on satiety	Control biscuits of commercial white wheat flour and biscuits made from corn containing 25 and 50% amylose	10 cm Visual Analog Scale	Increasing the level of wheat starch substitution with maize flour up to 50% resulted in a greater reduction in food intake at a subsequent meal	(195)
Meat and meat products	Effect of different textures of foods on satiation	Meat and meat replacer	100 mm visual analog scale	The negligible difference found for fullness and prospective consumption	(196)
	Acute satiety response and hormonal markers of appetite after consuming different types of meat	Chicken, pork and beef	Blood biomarkers; Ghrelin, PYY, Insulin, Glucose and CCK	Equated results for satiety response upon pork, beef, and chicken ingestion	(197)
	Postprandial glycemic and satiety response for fish protein hydrolysate in healthy adults	Boarfish protein hydrolysate (BPH) drink	Visual analog scale Ghrelin and leptin	No significant effect on biomarkers of satiety	(198)
	Effects of a beef-based meal compared to a calorie matched bean-based meal on appetite and food intake	Beef and beans	Visual analog scale	Beef-based meal with high protein and a bean-based meal with moderate protein and high fiber produced similar satiety	(199)

(Continued)

TABLE 1 (Continued)

Food groups	Aim of the study	Foods tested	Satiety measurement	Results	References
Fats and oils	Effect of fat saturation on satiety, hormone release, and food intake	Shea oil, canola oil and safflower oil	Visual analog scale	Triacylglycerols with unsaturated fatty acids increase satiety than with saturated fatty acids	(200)
	Coconut oil has less satiating properties than medium-chain triglyceride oil (MCT oil)	MCT oil, coconut oil and vegetable oil	Visual analog scale	MCT also increased fullness over the 3 h after breakfast compared to the vegetable and coconut oils	(201)
	The gastric emptying rate for specific food structures and impact on appetite suppression	Control meal (an emulsion of sunflower oil) and structured/active meal (gouda cheese and low-fat yogurt)	Visual analog scale	Active or structured meal significantly reduces hunger	(120)
	Effect of fat source on satiety	Canola and peanut oil muffins and canola, peanut oil, butter muffins	nine-point category scale	The slightly different satiating effect between saturated and monounsaturated fatty acids	(202)
	Effect of replacing breakfast with a high-fat drink	High fat (medium-chain triglycerides) meal replacement drink	Satiety labeled intensity magnitude	Increased satiety was reported in the afternoon after a high-fat meal replacement drink	(203)
Fruits and vegetables	Appetitive responses in lean and obese adults after ingesting fruits in solid vs. beverage forms	Solid fruit preload (red seedless grapes, dried apples, gala apple, raisins) and beverage fruit juice preloads	nine-point scale	Delaying time for hunger arousal was higher for solid fruit preloads	(40)
	Subjective assessment of hunger and fullness in healthy adults after ingesting orange pomace	Whole orange fruit, orange pomace and orange juice	Visual analog scale	The addition of orange pomace fiber to orange juice and whole fruit increased satiety in orange juice	(204)
	Using avocado as a test meal to test satiety	Whole avocado	Visual analog scale	Avocado-derived fat-fiber combination increased feelings of satiety and anorexigenic hormones PYY and GLP-1	(205)
	The effects of wild blueberries on satiety and glycemic control	Blueberry, blueberry juice, placebo beverage and control	Visual analog scale	Higher satisfaction when the whole blueberry treatment was consumed compared to the control	(206)
	Effects of Fresh Watermelon Consumption on the Acute Satiety Response	Watermelon and low-fat cookies as control snack	Visual analog scale and appetite regulating hormones	watermelon elicited robust satiety responses than cookies snacks. Watermelon also resulted in reduced leptin hormone and higher ghrelin	(207)
	Effect of fresh mango consumption on satiety	Fresh mango and iso-caloric low-fat cookies as control	Visual analog scale and appetite regulating hormones	Mangoes promote greater satiety and cookies did not reduce participants' desire to eat	(208)
	Influence of dietary carbohydrates and glycaemic response on subjective appetite and food intake	Potato, barley, glucose and placebo	Visual analog scale	Potatoes increased subjective satiety the most, followed by barley, then glucose	(209)
	Comparison of low glycemic index and high glycemic index potatoes about satiety in humans	Carisma® low Glycemic Index potatoes and Arizona high Glycemic Index potato varieties	Visual analog scale	No significant differences in the primary endpoint, satiety	(210)

(Continued)

TABLE 1 (Continued)

Food groups	Aim of the study	Foods tested	Satiety measurement	Results	References
	Effect of fenugreek fiber on satiety, blood glucose and insulin response	0, 4 and 8 g fenugreek extract beverage	Visual analog scale	Fenugreek fiber (8 g) significantly increased satiety	(211)
	Effect of capsaicin on satiety and energy intake	0.9 g of red Pepper in tomato juice, 0.9 g of red pepper in two capsules	Visual analog scale	The AUC for satiety increased, whereas the AUC for hunger decreased after capsaicin ingestion	(212)
	The effects of the fiber content and physical structure of carrots on satiety and subsequent intakes when eaten as part of a mixed meal	Whole carrots, blended carrots and carrot nutrients	Visual analog scale	Meals with whole carrots and blended carrots resulted in significantly higher satiety	(213)
Milk and milk products	The satiating potential of yogurt enriched with protein	Yogurt products	nine-point scale	Highest satiety scores for yogurt having added milk proteins	(214)
	Satiety and food intake after consuming different dairy products	Milk products	Visual analog scale	An increase in satiety has been observed after 500 ml of milk	(215)
	Effects of goat dairy and cow dairy-based breakfasts on satiety	Goat or cow dairy breakfast	Visual analog scale	The slightly higher satiating effect of goat dairy when compared to cow dairy	(216)
	Effect of casein-to-whey ratio in breakfast meals on postprandial satiety ratings	Milk with 80:20 or 40:60 casein-to-whey protein ratios	100 mm visual analog scale	The protein ratio did not significantly differ in satiety ratings after the second meal	(217)
	Satiety response of milk protein-derived peptides	Milk protein-derived peptides; sodium caseinate and a whey protein hydrolysate	Cumulative food intake	Sodium caseinate derived peptides suppressed appetite more than other peptides.	(218)
	Effects of cultured dairy and non-dairy products added to breakfast cereals on blood glucose control, satiation, satiety, and short-term food intake	Greek yogurt with granola, cultured coconut product with granola and water	100 mm visual analog scale	Intake of dairy suppresses the mean 2-h subjective appetite stronger compared to the non-dairy	(219)
	Milk protein fractions moderately extend the duration of satiety compared with carbohydrates	Milk proteins; casein, whey and their mixture	Energy intake at lunch	Compared with the control snack, proteins extended the duration of satiety with no difference between the protein groups	(220)
	Effects of low-fat milk consumption at breakfast on satiety and short-term energy intake	Low-fat milk, apple juice and water with breakfast	Visual analog scale	Obese children reported higher satiety score after drinking low-fat milk with breakfast	(221)

if it shows impact unrelated to energy density. Moreover, the satiety value for non-fermentable fiber is higher as compared to the fermentable ones (1). Consuming dietary fiber on a daily basis mainly in the form of salad can remarkably reduce the energy intake. Women ate pasta as a main course *ad libitum* on five different occasions, four times with a low-energy-dense salad (300 g, 100 kcal). The salad was provided 20 min before the pasta at two meals (once mandatory; once *ad libitum*), and the salad was presented with the pasta at two meals (once compulsory; once *ad libitum*). According to the findings, including a set amount of salad in the meal lowered energy intake by 11%, while eating a low-energy-dense salad before the main course increased vegetable consumption by 23%. Further results revealed that such an effect was correlated with serving size but was independent of the timing of intake (180).

Bioactive compounds

Certain bioactive ingredients in food can also influence satiety and subsequent energy intake. For example, caffeine has been found to influence energy balance as its prolonged consumption may help in weight loss. Similarly, the consumption of beverages containing caffeine or catechins in the form of green tea delays hungers arousal, thereby reducing energy intake (181). Likewise, the effects of capsaicin, green tea, and sweet pepper on hunger and appetite sensations along with energy intake have also been studied. The results revealed that a combination of green tea and capsaicin can effectively reduce energy intake in negative energy balance by enhancing satiety and suppressing hunger (182). In another study, a positive correlation between capsaicin and satiety has been found due to the release of satiety hormones (183).

Functional foods

The urge to discuss satiety and appetite regulation seems to be more than ever. Food manufacturers are always looking to provide items that people would be more willing to consume. Their goal is to provide goods that increase consumer appetite. Therefore, the increasing incidence of obesity and overweight issues is always attributed to the food industry (4). As a result, many food manufacturers throughout the world are changing the formulation of their products to develop products that can decrease appetite and calorie consumption, particularly in obese and overweight persons (184). Introducing functional foods in the market to suppress appetite requires consideration of crucial factors: efficacy, feasibility, acceptability and effective size (185). Some substances have an indirect effect on appetite while each product must also be feasible in terms of the equipment needed for production, processing, and storage. Additionally, when designing such products, consideration must be given to the magnitude of each compound or the total of compounds' effects on hunger (186). Functional food is included in products that make performance-related claims and claims to decrease appetite. In this way, they influence the body's function or feeling of appetite and may modulate it. Most producers frequently misuse these items, which leads to consumer confusion. Any claim of reduced appetite must be supported by credible, scientific evidence. Long-term human studies should support any claims of weight loss that may follow from using appetite suppressants. Any claim that a substance decreases appetite should also be presented in comparison. As a result, two groups—one control and one intervention must be chosen, their respective levels of appetite reduction must be assessed, and confounding variables must be taken into account (187). The price of proteins and fibers is typically substantially greater than that of other ingredients used in the food industry, and they are typically among the key components of most products planned and produced to lower hunger. Such products will cost more since a combination of vitamins and minerals will be added to them to prevent malnutrition. Therefore, it can be acknowledged that the people or groups with high social and economic standing are the target market for the majority of functional foods, which is seen as one of their limitations (188).

Satiety response of food groups

Food groups such as cereals, meat, fat, fruits and vegetables, and dairy products (Table 1) vary in their ability to satisfy hunger as there are multiple putative mechanisms by which food components send signals to the brain, which affect the gut and induce satiety.

Variation in satiety responses among these five basic food groups exists since they offer different macronutrient compositions as cereals are high in carbohydrates, while meat and meat products are rich sources of proteins. Likewise, fruits and vegetables provide soluble and insoluble dietary fiber. Apart, satiety index scores for a variety of isocaloric foods have also been developed (27). Among all food groups, fruits and vegetables received the highest satiety scores, and refined cereal products gained the lowest satiety scores. Considering many internal and external factors, the food matrix may particularly affect satiation and satiety due to its interaction with the gut at various levels from ingestion to absorption along with other related components being discussed in the review.

Conclusions

Satiety is a complex and dynamic process that can be modulated while attempting to achieve improved fullness and reduce caloric intake. Different strategies for individual health goals are often applied to regulate the underlying factors affecting food intake from the cephalic to gastric phase. The meals high in protein, with larger portion sizes and lower calorie density, as well as higher viscosity of digesta (either solid or semisolid), stomach emptying and controlling hedonic hunger improve the satiety response, whereas satiation is enhanced with the high-fat foods. Furthermore, the post-digestive or post-absorptive response of foods greatly affects satiation or satiety through gut-brain signaling and energy homeostasis. Besides, body composition (more leptin in females), specific meal size in different cultural cuisines, increased food mastication, consistent physical activity, and overexpression of anorexigenic hormones triggered by the SCFA produced by the gut microbiome upon dietary fiber consumption are just a few of the personal factors that may lead to reduced food intake or improved satiety signaling. Since eating behaviors are heritable, variations in physical activity, sleep, and circadian rhythm all together play an important role in explicating an individual's food intake patterns. The current review has thus examined the totality of the evidence for several personal and food-related factors that may influence the consumption of foods or in turn satiety eliciting response. However, further interventions focusing on the systemic impact of nutrients (e.g., *via* gut microbiota modulation) need to be designed for a long enough time to better understand nutrient-induced satiety and weight regulation.

Author contributions

Conceptualization: FM and AR. Investigation: FM and RA. Data curation: MA and FM. Writing—original draft preparation: FM, AR, and MS. Writing—review and editing: FM, WA, AH, CS, and MR. Visualization: AVR, MS, and RA. Provided guidance in the manuscript revision: AVR and RA. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Slavin J, Green H. Dietary fibre and satiety. *Nutr Bull.* (2007) 32:32–42. doi: 10.1111/j.1467-3010.2007.00603.x

2. Tremblay A, Bellisle F. Nutrients, satiety, and control of energy intake. *Appl Physiol Nutr Metab.* (2015) 40:971–9. doi: 10.1139/apnm-2014-0549

3. Fiszman S, Varela P, Díaz P, Linares MB, Garrido M. What is satiating? Consumer perceptions of satiating foods and expected satiety of protein-based meals. *Int Food Res J.* (2014) 62:551–60. doi: 10.1016/j.foodres.2014.03.065

4. Hetherington M, Cunningham K, Dye L, Gibson E, Gregersen N, Halford J, et al. Potential benefits of satiety to the consumer: scientific considerations. *Nutr Res Rev.* (2013) 26:22–38. doi: 10.1017/S0954422413000012

5. Basit A, Shera AS. Prevalence of metabolic syndrome in Pakistan. *Metab Syndr Relat Disord.* (2008) 6:171–5. doi: 10.1089/met.2008.0005

6. Soong YY, Quek RYC, Henry CJ. Glycemic potency of muffins made with wheat, rice, corn, oat and barley flours: a comparative study between *in vivo* and *in vitro*. *Eur J Nutr.* (2015) 54:1281–5. doi: 10.1007/s00394-014-0806-9

7. Guyenet SJ, Schwartz MW. Regulation of food intake, energy balance, and body fat mass: implications for the pathogenesis and treatment of obesity. *J Clin Endocr.* (2012) 97:745–55. doi: 10.1210/jc.2011-2525

8. Drapeau V, King N, Hetherington M, Doucet E, Blundell J, Tremblay A. Appetite sensations and satiety quotient: predictors of energy intake and weight loss. *Appetite.* (2007) 48:159–66. doi: 10.1016/j.appet.2006.08.002

9. Hardcastle SJ, Thøgersen-Ntoumani C, Chatzisarantis NL. Food choice and nutrition: a social psychological perspective. *Nutrients.* (2015) 7:8712–5. doi: 10.3390/nu7105424

10. Gibbons C, Blundell J. Appetite regulation and physical activity: an energy balance perspective. *Hamdan Med J.* (2015) 212:1–20. doi: 10.7707/hmj.431

11. Davidson TL, Jones S, Roy M, Stevenson RJ. The cognitive control of eating and body weight: it's more than what you "think". *Front Psychol.* (2019) 10:62–83. doi: 10.3389/fpsyg.2019.00062

12. Krop EM, Hetherington MM, Nekitsing C, Miquel S, Postelnicu L, Sarkar A. Influence of oral processing on appetite and food intake–A systematic review and meta-analysis. *Appetite.* (2018) 125:253–69. doi: 10.1016/j.appet.2018.01.018

13. Hopkins M, Blundell J, Halford J, King N, Finlayson G. *The Regulation of Food Intake In Humans. Endotext.* (2016).

14. Ni D, Smyth HE, Cozzolino D, Gidley MJ. Integrating effects of human physiology, psychology, and individual variations on satiety–An exploratory study. *Front Nutr.* (2022) 9. doi: 10.3389/fnut.2022.872169

15. Derks IP, Sijbrands EJ, Wake M, Qureshi F, Van Der Ende J, Hillegers MH, et al. Eating behavior and body composition across childhood: a prospective cohort study. *Int J Behav Nutr Phys Act.* (2018) 15:1–9. doi: 10.1186/s12966-018-0725-x

16. Mccrickerd K, Forde C. Sensory influences on food intake control: moving beyond palatability. *Obes Rev.* (2016) 17:18–29. doi: 10.1111/obr.12340

17. Rolls ET. Taste, olfactory, and food texture processing in the brain, and the control of food intake. *Physiol Behav.* (2005) 85:45–56. doi: 10.1016/j.physbeh.2005.04.012

18. Cooper DN, Martin RJ, Keim NL. Does whole grain consumption alter gut microbiota and satiety? In: *Healthcare*. Basel, Switzerland: Multidisciplinary Digital Publishing Institute (2015). p. 364–92. doi: 10.3390/healthcare3020364

19. Williams RA, Roe LS, Rolls BJ. Assessment of satiety depends on the energy density and portion size of the test meal. *Obesity.* (2014) 22:318–24. doi: 10.1002/oby.20589

20. Stribitcaia E, Evans CE, Gibbons C, Blundell J, Sarkar A. Food texture influences on satiety: systematic review and meta-analysis. *Sci Rep.* (2020) 10:1–18. doi: 10.1038/s41598-020-69504-y

21. Adam CL, Williams PA, Garden KE, Thomson LM, Ross AW. Dose-dependent effects of a soluble dietary fibre (pectin) on food intake, adiposity, gut hypertrophy and gut satiety hormone secretion in rats. *PLoS ONE.* (2015) 10:e0115438. doi: 10.1371/journal.pone.0115438

22. Forde CG. Measuring satiation and satiety. In: *Methods in Consumer Research, Vol 2.* Amsterdam: Elsevier Science & Technology (2018) p. 151–82. doi: 10.1016/B978-0-08-101743-2.00007-8

23. Blundell J, De Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite control: methodological aspects of the evaluation of foods. *Obes Rev.* (2010) 11:251–70. doi: 10.1111/j.1467-789X.2010.00714.x

24. Van Kleef E, Shimizu M, Wansink B. Just a bite: Considerably smaller snack portions satisfy delayed hunger and craving. *Food Qual Prefer.* (2013) 27:96–100. doi: 10.1016/j.foodqual.2012.06.008

25. Brum JM, Gibb RD, Peters JC, Mattes RD. Satiety effects of psyllium in healthy volunteers. *Appetite.* (2016) 105:27–36. doi: 10.1016/j.appet.2016.04.041

26. Berti C, Riso P, Brusamolino A, Porrini M. Effect on appetite control of minor cereal and pseudocereal products. *Br J Nutr.* (2005) 94:850–8. doi: 10.1079/BJN20051563

27. Holt SH, Brand Miller J, Petocz P, Farmakalidis E, A. satiety index of common foods. *Eur J Clin Nutr.* (1995) 49:675–90.

28. Flint A, Raben A, Blundell J, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes.* (2000) 24:38–48. doi: 10.1038/sj.ijo.0801083

29. Gibbons C, Hopkins M, Beaulieu K, Oustric P, Blundell JE. Issues in measuring and interpreting human appetite (satiety/satiation) and its contribution to obesity. *Curr Obes Rep.* (2019) 8:77–87. doi: 10.1007/s13679-019-00340-6

30. Green S, Delargy H, Joanes D, Blundell J, A. satiety quotient: a formulation to assess the satiating effect of food. *Appetite.* (1997) 29:291–304. doi: 10.1006/appe.1997.0096

31. Ni D, Smyth HE, Gidley MJ, Cozzolino D. Shedding light on human tissue (*in vivo*) to predict satiation, satiety, and food intake using near infrared reflectance spectroscopy: A preliminary study. *Innov Food Sci Emer.* (2022) 103033. doi: 10.1016/j.ifset.2022.103033

32. Posovszky C, Wabitsch M. Regulation of appetite, satiation, and body weight by enteroendocrine cells. Part 1: characteristics of enteroendocrine cells and their capability of weight regulation. *Horm Res Paediatr.* (2015) 83:1–10. doi: 10.1159/000368898

33. De Graaf C, Blom WA, Smeets PA, Stafleu A, Hendriks HF. Biomarkers of satiation and satiety. *Am J Clin Nutr.* (2004) 79:946–61. doi: 10.1093/ajcn/79.6.946

34. Aziz Q. Brain–gut interactions in the regulation of satiety: new insights from functional brain imaging. *Gut.* (2012) 61:1521–2. doi: 10.1136/gutjnl-2012-302368

35. Lenard NR, Berthoud HR. Central and peripheral regulation of food intake and physical activity: pathways and genes. *Obesity.* (2008) 16:S11–22. doi: 10.1038/oby.2008.511

36. De Araujo IE. Expanding the brain glucosensing territory. *Cell Metab.* (2014) 20:933–5. doi: 10.1016/j.cmet.2014.11.013

37. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota–gut–brain axis in obesity. *Lancet Gastroenterol Hepatol.* (2017) 2:747–56. doi: 10.1016/S2468-1253(17)30147-4

38. Pizarroso NA, Fuciños P, Gonçalves C, Pastrana L, Amado IR, A. review on the role of food-derived bioactive molecules and the microbiota–gut–brain axis in satiety regulation. *Nutrients.* (2021) 13:632. doi: 10.3390/nu13020632

39. A. Parnell JA, Reimer R. Prebiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome. *Gut Microbes.* (2012) 3:29-34. doi: 10.4161/gmic.19246

40. Houchins JA, Tan S-Y, Campbell WW, Mattes RD. Effects of fruit and vegetable, consumed in solid vs. beverage forms, on acute and chronic appetitive responses in lean and obese adults. *Int J Obes.* (2013) 37:1109–15. doi: 10.1038/ijo.2012.183

41. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr.* (2018) 57:1–24. doi: 10.1007/s00394-017-1445-8

42. Cornejo-Pareja I, Muñoz-Garach A, Clemente-Postigo M, Tinahones FJ. Importance of gut microbiota in obesity. *Eur J Clin Nutr.* (2019) 72:26–37. doi: 10.1038/s41430-018-0306-8

43. Duggal P, Guo X, Haque R, Peterson KM, Ricklefs S, Mondal D, et al. A mutation in the leptin receptor is associated with Entamoeba histolytica infection in children. *J Clin Invest.* (2011) 121:1191–8. doi: 10.1172/JCI45294

44. Guo X, Roberts MR, Becker SM, Podd B, Zhang Y, Chua S, et al. Leptin signaling in intestinal epithelium mediates resistance to enteric infection by Entamoeba histolytica. *Mucosal Immunol.* (2011) 4:294–303. doi: 10.1038/mi.2010.76

45. Rajala MW, Patterson CM, Opp JS, Foltin SK, Young VB, Myers MG. Leptin acts independently of food intake to modulate gut microbial composition in male mice. *Endocrinology.* (2014) 155:748–57. doi: 10.1210/en.2013-1085

46. Schéle E, Grahnemo L, Anesten F, Hallén A, Bäckhed F, Jansson J-O. The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (GCG) and brain-derived neurotrophic factor (BDNF) in the central nervous system. *Endocrinology.* (2013) 154:3643–51. doi: 10.1210/en.2012-2151

47. Socol CT, Chira A, Martinez-Sanchez MA, Nuñez-Sanchez MA, Maerescu CM, Mierlita D, et al. Leptin signaling in obesity and colorectal cancer. *Int J Mol Sci.* (2022) 23:4713. doi: 10.3390/ijms23094713

48. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, et al. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes.* (2011) 60:2775–86. doi: 10.2337/db11-0227

49. Ibrügger S, Vigsnæs LK, Blennow A, Škuflić D, Raben A, Lauritzen L, et al. Second meal effect on appetite and fermentation of wholegrain rye foods. *Appetite.* (2014) 80:248–56. doi: 10.1016/j.appet.2014.05.026

50. Rosén LA, Östman EM, Björck IM. Effects of cereal breakfasts on postprandial glucose, appetite regulation and voluntary energy intake at a subsequent standardized lunch; focusing on rye products. *Nutr J.* (2011) 10:1–11. doi: 10.1186/1475-2891-10-7

51. Jeong W, Jang S-I. Associations between meal companions and obesity in South Korean adults. *Int J Environ Res Public Health.* (2020) 17:2697. doi: 10.3390/ijerph17082697

52. Teo PS, Forde CG. The Impact of eating rate on energy intake, body composition, and health. *Handbook of Eating and Drinking: Interdisciplinary Perspectives*. New York, NY: Springer International Publishing (2020) 715–40. doi: 10.1007/978-3-030-14504-0_120

53. Haghighian-Roudsari A, Milani-Bonab A, Mirzay-Razaz J, Vedadhir A. Food choice as a social problem: a reflection on the socio-cultural determinants of food choice. *Commun Health.* (2018) 5:291–302.

54. Vaughn AE, Ward DS, Fisher JO, Faith MS, Hughes SO, Kremers SP. Fundamental constructs in food parenting practices: a content map to guide future research. *Nutr Rev.* (2016) 74:98–117. doi: 10.1093/nutrit/nuv061

55. Monterrosa EC, Frongillo EA, Drewnowski A, De Pee S, Vandevijvere S. Sociocultural influences on food choices and implications for sustainable healthy diets. *Food Nutr Bull.* (2020) 41:59S—73S. doi: 10.1177/0379572120975874

56. Blundell J, Goodson S, Halford J. Regulation of appetite: role of leptin in signalling systems for drive and satiety. *Int J Obes Relat Metab Disord.* (2001) 25:29–34. doi: 10.1038/sj.ijo.0801693

57. Davidson T, Kanoski SE, Walls EK, Jarrard LE. Memory inhibition and energy regulation. *Physiol Behav.* (2005) 86:731–46. doi: 10.1016/j.physbeh.2005.09.004

58. Veasey RC, Haskell-Ramsay CF, Kennedy DO, Tiplady B, Stevenson EJ. The effect of breakfast prior to morning exercise on cognitive performance, mood and appetite later in the day in habitually active women. *Nutrients.* (2015) 7:5712–32. doi: 10.3390/nu7075250

59. Mittal D, Stevenson RJ, Oaten MJ, Miller LA. Snacking while watching TV impairs food recall and promotes food intake on a later TV free test meal. *Appl Cogn Psychol.* (2011) 25:871–7. doi: 10.1002/acp.1760

60. Blass EM, Anderson DR, Kirkorian HL, Pempek TA, Price I, Koleini MF. On the road to obesity: television viewing increases intake of high-density foods. *Physiol Behav.* (2006) 88:597–604. doi: 10.1016/j.physbeh.2006.05.035

61. Bellissimo N, Pencharz PB, Thomas SG, Anderson GH. Effect of television viewing at mealtime on food intake after a glucose preload in boys. *Pediatr Res.* (2007) 61:745–9. doi: 10.1203/pdr.0b013e3180536591

62. Mattes MZ, Vickers ZM. Better-liked foods can produce more satiety. *Food Qual Prefer.* (2018) 64:94–102. doi: 10.1016/j.foodqual.2017.10.012

63. Akyol A, Ayaz A, Inan-Eroglu E, Cetin C, Samur G. Impact of three different plate colours on short-term satiety and energy intake: a randomized controlled trial. *Nutr J.* (2018) 17:1–8. doi: 10.1186/s12937-018-0350-1

64. Bédard A, Hudon A-M, Drapeau V, Corneau L, Dodin S, Lemieux S. Gender differences in the appetite response to a satiating diet. *J Obes.* (2015). doi: 10.1155/2015/140139

65. Cornier M-A, Salzberg AK, Endly DC, Bessesen DH, Tregellas JR. Sex-based differences in the behavioral and neuronal responses to food. *Physiol Behav.* (2010) 99:538–43. doi: 10.1016/j.physbeh.2010.01.008

66. Shi H, Clegg D. Sex differences in the regulation of body weight. *Physiol Behav.* (2009) 97:199–204. doi: 10.1016/j.physbeh.2009.02.017

67. Beckman LM, Beckman TR, Earthman CP. Changes in gastrointestinal hormones and leptin after Roux-en-Y gastric bypass procedure: a review. *J Am Diet Assoc.* (2010) 110:571–84. doi: 10.1016/j.jada.2009.12.023

68. Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, et al. Leptin and obesity: role and clinical implication. *Front Endocrinol.* (2021) 12:585887. doi: 10.3389/fendo.2021.585887

69. Chrysafi P, Perakakis N, Farr OM, Stefanakis K, Peradze N, Sala-Vila A, et al. Leptin alters energy intake and fat mass but not energy expenditure in lean subjects. *Nat Commun.* (2020) 11:1–15. doi: 10.1038/s41467-020-18885-9

70. Donini LM, Savina C, Cannella C. Eating habits and appetite control in the elderly: the anorexia of aging. *Int Psychogeriatr.* (2003) 15:73–87. doi: 10.1017/S1041610203008779

71. Rolls BJ, Mcdermott TM. Effects of age on sensory-specific satiety. *Am J Clin Nutr.* (1991) 54:988–96. doi: 10.1093/ajcn/54.6.988

72. Bellisle F, Guy-Grand B, Le Magnen J. Chewing and swallowing as indices of the stimulation to eat during meals in humans: effects revealed by the edogram method and video recordings. *Neurosci Biobehav Rev.* (2000) 24:223–8. doi: 10.1016/S0149-7634(99)00075-5

73. Cassady BA, Hollis JH, Fulford AD, Considine RV, Mattes RD. Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. *Am J Clin Nutr.* (2009) 89:794–800. doi: 10.3945/ajcn.2008.26669

74. Park E, Edirisinghe I, Inui T, Kergoat S, Kelley M, Burton-Freeman B. Short-term effects of chewing gum on satiety and afternoon snack intake in healthy weight and obese women. *Physiol Behav.* (2016) 159:64–71. doi: 10.1016/j.physbeh.2016.03.002

75. Ioakimidis I, Zandian M, Eriksson-Marklund L, Bergh C, Grigoriadis A, Södersten P. Description of chewing and food intake over the course of a meal. *Physiol Behav.* (2011) 104:761–9. doi: 10.1016/j.physbeh.2011.07.021

76. Smit HJ, Kemsley EK, Tapp HS, Henry CJK. Does prolonged chewing reduce food intake? Fletcherism revisited. *Appetite.* (2011) 57:295–8. doi: 10.1016/j.appet.2011.02.003

77. Higgs S, Jones A. Prolonged chewing at lunch decreases later snack intake. *Appetite.* (2013) 62:91–5. doi: 10.1016/j.appet.2012.11.019

78. Scisco JL, Muth ER, Dong Y, Hoover AW. Slowing bite-rate reduces energy intake: an application of the bite counter device. *J Am Diet Assoc.* (2011) 111:1231–5. doi: 10.1016/j.jada.2011.05.005

79. Hollis JH. The effect of mastication on food intake, satiety and body weight. *Physiol Behav.* (2018) 193:242–5. doi: 10.1016/j.physbeh.2018.04.027

80. Tsofliou F, Pitsiladis Y, Malkova D, Wallace A, Lean M. Moderate physical activity permits acute coupling between serum leptin and appetite–satiety measures in obese women. *Int J Obes.* (2003) 27:1332–9. doi: 10.1038/sj.ijo.0802406

81. Blundell J, Gibbons C, Caudwell P, Finlayson G, Hopkins M. Appetite control and energy balance: impact of exercise. *Obes Rev.* (2015) 16:67–76. doi: 10.1111/obr.12257

82. Beaulieu K, Hopkins M, Blundell J, Finlayson G. Impact of physical activity level and dietary fat content on passive overconsumption of energy in non-obese adults. *Int J Behav Nutr Phys Act.* (2017) 14:14. doi: 10.1186/s12966-017-0473-3

83. Riondino S, Roselli M, Palmirotta R, Della-Morte D, Ferroni P, Guadagni F. Obesity and colorectal cancer: role of adipokines in tumor initiation and progression. *World J Gastroenterol.* (2014) 20:5177. doi: 10.3748/wjg.v20.i18.5177

84. Bharath LP, Choi WW, Cho J-M, Skobodzinski AA, Wong A, Sweeney TE, et al. Combined resistance and aerobic exercise training reduces insulin resistance and central adiposity in adolescent girls who are obese: randomized clinical trial. *Eur J Appl Physiol.* (2018) 118:1653–60. doi: 10.1007/s00421-018-3898-8

85. Kim S-W, Jung W-S, Park W, Park H-Y. Twelve weeks of combined resistance and aerobic exercise improves cardiometabolic biomarkers and enhances red blood cell hemorheological function in obese older men: a randomized controlled trial. *Int J Environ Res Public Health.* (2019) 16:5020. doi: 10.3390/ijerph16245020

86. Nunes PR, Martins FM, Souza AP, Carneiro MA, Orsatti CL, Michelin MA, et al. Effect of high-intensity interval training on body composition and inflammatory markers in obese postmenopausal women: a randomized controlled trial. *Menopause.* (2019) 26:256–64. doi: 10.1097/GME.0000000000001207

87. Ness KM, Strayer SM, Nahmod NG, Schade MM, Chang A-M, Shearer GC, et al. Four nights of sleep restriction suppress the postprandial lipemic response and decrease satiety. *J Lipid Res.* (2019) 60:1935–45. doi: 10.1194/jlr.P094375

88. Mcneil J, Drapeau V, Gallant A, Tremblay A, Doucet É, Chaput J-P. Short sleep duration is associated with a lower mean satiety quotient in overweight and obese men. *Eur J Clin Nutr.* (2013) 67:1328–30. doi: 10.1038/ejcn.2013.204

89. Landis AM, Parker KP, Dunbar SB. Sleep, hunger, satiety, food cravings, and caloric intake in adolescents. *J Nurs Scholarsh.* (2009) 41:115–23. doi: 10.1111/j.1547-5069.2009.01262.x

90. Nedeltcheva AV, Kilkus JM, Imperial J, Kasza K, Schoeller DA, Penev PD. Sleep curtailment is accompanied by increased intake of calories from snacks. *Am J Clin Nutr.* (2009) 89:126–33. doi: 10.3945/ajcn.2008.26574

91. Markwald RR, Melanson EL, Smith MR, Higgins J, Perreault L, Eckel RH, et al. Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. *Proc Nat Acad Sci.* (2013) 110:5695–700. doi: 10.1073/pnas.1216951110

92. Mchill AW, Melanson EL, Higgins J, Connick E, Moehlman TM, Stothard ER, et al. Impact of circadian misalignment on energy metabolism during simulated nightshift work. *Proc Nat Acad Sci.* (2014) 111:17302–7. doi: 10.1073/pnas.1412021111

93. Morris CJ, Garcia JI, Myers S, Yang JN, Trienekens N, Scheer FA. The human circadian system has a dominating role in causing the morning/evening difference in diet-induced thermogenesis. *Obesity.* (2015) 23:2053–8. doi: 10.1002/oby.21189

94. Mchill AW, Hull JT, Mcmullan CJ, Klerman EB. Chronic insufficient sleep has a limited impact on circadian rhythmicity of subjective hunger and awakening fasted metabolic hormones. *Front Endocrinol.* (2018) 9:319–29. doi: 10.3389/fendo.2018.00319

95. Mchill AW, Hull JT, Klerman EB. Chronic circadian disruption and sleep restriction influence subjective hunger, appetite, and food preference. *Nutrients.* (2022) 14:1800. doi: 10.3390/nu14091800

96. Chrobok L, Klich JD, Sanetra AM, Jeczmien-Lazur JS, Pradel K, Palus-Chramiec K, et al. Rhythmic neuronal activities of the rat nucleus of the solitary tract are impaired by high-fat diet–implications for daily control of satiety. *J Physiol.* (2022) 600:751–67. doi: 10.1113/JP281838

97. Grimm ER, Steinle NI. Genetics of eating behavior: established and emerging concepts. *Nutr Rev.* (2011) 69:52–60. doi: 10.1111/j.1753-4887.2010.00361.x

98. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature.* (2015) 518:197–206. doi: 10.1038/nature14177

99. Suarez AN, Liu CM, Cortella AM, Noble EE, Kanoski SE. Ghrelin and orexin interact to increase meal size through a descending hippocampus to hindbrain signaling pathway. *Biol Psychiatry.* (2020) 87:1001–11. doi: 10.1016/j.biopsych.2019.10.012

100. Domingo-Rodriguez L, Ruiz De Azua I, Dominguez E, Senabre E, Serra I, Kummer S, et al. A specific prelimbic-nucleus accumbens pathway controls resilience versus vulnerability to food addiction. *Nat Commun.* (2020) 11:1–16. doi: 10.1038/s41467-020-14458-y

101. Ndiaye FK, Huyvaert M, Ortalli A, Canouil M, Lecoeur C, Verbanck M, et al. The expression of genes in top obesity-associated loci is enriched in insula and substantia nigra brain regions involved in addiction and reward. *Int J Obes.* (2020) 44:539–43. doi: 10.1038/s41366-019-0428-7

102. Wright H, Li X, Fallon NB, Crookall R, Giesbrecht T, Thomas A, et al. Differential effects of hunger and satiety on insular cortex and hypothalamic functional connectivity. *Eur J Neurosci.* (2016) 43:1181–9. doi: 10.1111/ejn.13182

103. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *Am J Clin Nutr.* (2005) 81:1298–306. doi: 10.1093/ajcn/81.6.1298

104. Yang Q, Xiao T, Guo J, Su Z. Complex relationship between obesity and the fat mass and obesity locus. *Int J Biol Sci.* (2017) 13:615. doi: 10.7150/ijbs.17051

105. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science.* (2007) 316:889–94. doi: 10.1126/science.1141634

106. Magno FCCM, Guarana HC, Fonseca ACP, Cabello GMK, Carneiro JRI, Pedrosa AP, et al. Influence of FTO rs9939609 polymorphism on appetite, ghrelin, leptin, IL6, TNFα levels, and food intake of women with morbid obesity. *Diabetes Metab Syndr Obes.* (2018) 11:199. doi: 10.2147/DMSO.S154978

107. Szalanczy AM, Key C-CC, Woods LCS. Genetic variation in satiety signaling and hypothalamic inflammation: merging fields for the study of obesity. *J Nutr Biochem.* (2022) 101:108928. doi: 10.1016/j.jnutbio.2021.108928

108. Löffler MC, Betz MJ, Blondin DP, Augustin R, Sharma AK, Tseng Y-H, et al. Challenges in tackling energy expenditure as obesity therapy: from preclinical models to clinical application. *Mol Metab.* (2021) 51:101237. doi: 10.1016/j.molmet.2021.101237

109. Meule A, Lutz A, Vögele C, Kübler A. Food cravings discriminate differentially between successful and unsuccessful dieters and non-dieters. Validation of the Food Cravings Questionnaires in German. *Appetite.* (2012) 58:88–97. doi: 10.1016/j.appet.2011.09.010

110. Reents J, Seidel A-K, Wiesner CD, Pedersen A. The effect of hunger and satiety on mood-related food craving. *Front Psychol.* (2020) 11:568908. doi: 10.3389/fpsyg.2020.568908

111. Boland M. Human digestion–a processing perspective. *J Sci Food Agric.* (2016) 96:2275–83. doi: 10.1002/jsfa.7601

112. Guo Q, Ye A, Singh H, Rousseau D. Destructuring and restructuring of foods during gastric digestion. *Compr Rev Food Sci.* (2020) 19:1658–79. doi: 10.1111/1541-4337.12558

113. Somaratne G, Nau F, Ferrua MJ, Singh J, Ye A, Dupont D, et al. Characterization of egg white gel microstructure and its relationship with pepsin diffusivity. *Food Hydrocoll.* (2020) 98:105258. doi: 10.1016/j.foodhyd.2019.105258

114. Santos-Hernández M, Miralles B, Amigo L, Recio I. Intestinal signaling of proteins and digestion-derived products relevant to satiety. *J Agric Food Chem.* (2018) 66:10123–31. doi: 10.1021/acs.jafc.8b02355

115. Zoon HF, De Graaf C, Boesveldt S. Food odours direct specific appetite. *Foods.* (2016) 5:12. doi: 10.3390/foods5010012

116. Hendriks-Hartensveld AE, Rolls BJ, Cunningham PM. Nederkoorn C. Does labelling a food as 'light'vs'filling'influence intake and sensory-specific satiation? *Appetite.* (2022) 171:105916. doi: 10.1016/j.appet.2022.105916

117. Dhillon J, Running CA, Tucker RM, Mattes RD. Effects of food form on appetite and energy balance. *Food Qual Prefer.* (2016) 48:368–75. doi: 10.1016/j.foodqual.2015.03.009

118. Jones LV, Jones KM, Hensman C, Bertuch R, Mcgee TL, Dixon JB. Solid vs. liquid—satiety study in well-adjusted lap-band patients. *Obes Surg.* (2013) 23:1266–72. doi: 10.1007/s11695-013-0897-z

119. Mattes RD, Rothacker D. Beverage viscosity is inversely related to postprandial hunger in humans. *Physiol Behav.* (2001) 74:551–7. doi: 10.1016/S0031-9384(01)00597-2

120. Mackie AR, Rafiee H, Malcolm P, Salt L, Van Aken G. Specific food structures supress appetite through reduced gastric emptying rate. *Am J Physiol Gastrointest Liver Physiol.* (2013) 304:G1038–43. doi: 10.1152/ajpgi.00060.2013

121. Lett AM, Norton JE, Yeomans MR. Emulsion oil droplet size significantly affects satiety: A pre-ingestive approach. *Appetite.* (2016) 96:18–24. doi: 10.1016/j.appet.2015.08.043

122. Isaksson H, Rakha A, Andersson R, Fredriksson H, Olsson J, Åman P. Rye kernel breakfast increases satiety in the afternoon-an effect of food structure. *Nutr J.* (2011) 10:31. doi: 10.1186/1475-2891-10-31

123. Brand JC, Nicholson PL, Thorburn AW, Truswell AS. Food processing and the glycemic index. *Am J Clin Nutr.* (1985) 42:1192–6. doi: 10.1093/ajcn/42.6.1192

124. Rebello CJ, Johnson WD, Martin CK, Xie W, O'Shea M, Kurilich A, et al. Acute effect of oatmeal on subjective measures of appetite and satiety compared to a ready-to-eat breakfast cereal: a randomized crossover trial. *J Am Coll Nutr* . (2013) 32:272-9. doi: 10.1080/07315724.2013.816614

125. Brunstrom JM. Mind over platter: pre-meal planning and the control of meal size in humans. *Int J Obes.* (2014) 38:S9–S12. doi: 10.1038/ijo.2014.83

126. Holt SH, Brand-Miller JC, Stitt PA. The effects of equal-energy portions of different breads on blood glucose levels, feelings of fullness and subsequent food intake. *J Am Diet Assoc.* (2001) 101:767–73. doi: 10.1016/S0002-8223(01)00192-4

127. Rolls BJ, Roe LS, Meengs JS. Reductions in portion size and energy density of foods are additive and lead to sustained decreases in energy intake. *Am J Clin Nutr.* (2006) 83:11–7. doi: 10.1093/ajcn/83.1.11

128. Rolls BJ, Engell D, Birch LL. Serving portion size influences 5-year-old but not 3-year-old children's food intakes. *J Acad Nutr Diet.* (2000) 100:232. doi: 10.1016/S0002-8223(00)00070-5

129. Fisher JO, Rolls BJ, Birch LL. Children's bite size and intake of an entree are greater with large portions than with age-appropriate or self-selected portions. *Am J Clin Nutr.* (2003) 77:1164–70. doi: 10.1093/ajcn/77.5.1164

130. Snetselaar LG, De Jesus JM, Desilva DM, Stoody EE. Dietary guidelines for americans, 2020–2025: understanding the scientific process, guidelines, and key recommendations. *Nutr Today.* (2021) 56:287. doi: 10.1097/NT.0000000000000512

131. Gelberg L, Rico MW, Herman DR, Belin TR, Chandler M, Ramirez E, et al. Comparative effectiveness trial comparing MyPlate to calorie counting for mostly low-income Latino primary care patients of a federally qualified community health center: study design, baseline characteristics. *BMC Public Health.* (2019) 19:1–21. doi: 10.1186/s12889-019-7294-z

132. Duncan KH, Bacon JA, Weinsier RL. The effects of high and low energy density diets on satiety, energy intake, and eating time of obese and nonobese subjects. *Am J Clin Nutr.* (1983) 37:763–7. doi: 10.1093/ajcn/37.5.763

133. Latner J, Schwartz M. The effects of a high-carbohydrate, high-protein or balanced lunch upon later food intake and hunger ratings. *Appetite.* (1999) 33:119–28. doi: 10.1006/appe.1999.0237

134. Feinle C, O'Donovan D, Horowitz M. Carbohydrate and satiety. *Nutr Rev.* (2002) 60:155–69. doi: 10.1301/002966402320243241

135. Anderson GH, Woodend D. Consumption of sugars and the regulation of short-term satiety and food intake. *Am J Clin Nutr.* (2003) 78:843S—9S. doi: 10.1093/ajcn/78.4.843S

136. Mayer J. Glucostatic mechanism of regulation of food intake. *N Engl J Med.* (1953) 249:13–6. doi: 10.1056/NEJM195307022490104

137. Alfenas RC, Mattes RD. Influence of glycemic index/load on glycemic response, appetite, and food intake in healthy humans. *Diabetes Care.* (2005) 28:2123–9. doi: 10.2337/diacare.28.9.2123

138. Juanola-Falgarona M, Salas-Salvadó J, Ibarrola-Jurado N, Rabassa-Soler A, Díaz-López A, Guasch-Ferré M, et al. Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. *Am J Clin Nutr.* (2014) 100:27–35. doi: 10.3945/ajcn.113.081216

139. Westerterp-Plantenga MS, Lemmens SG, Westerterp KR. Dietary protein–its role in satiety, energetics, weight loss and health. *Br J Nutr.* (2012) 108:S105–12. doi: 10.1017/S0007114512002589

140. Veldhorst M, Nieuwenhuizen A, Hochstenbach-Waelen A, Westerterp K, Engelen M, Brummer R, et al. Effects of high or normal casein-, soy-, or whey with or without GMP-protein breakfasts on satiety,'satiety'hormones, and plasma amino acid responses. *Appetite.* (2007) 49:336. doi: 10.1016/j.appet.2007.03.206

141. Veldhorst M, Smeets A, Soenen S, Hochstenbach-Waelen A, Hursel R, Diepvens K, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav.* (2008) 94:300–7. doi: 10.1016/j.physbeh.2008.01.003

142. Yang D, Liu Z, Yang H, Jue Y. Acute effects of high-protein vs. normal-protein isocaloric meals on satiety and ghrelin. *Eur J Nutr.* (2014) 53:493–500. doi: 10.1007/s00394-013-0552-4

143. Veldhorst MA, Westerterp KR, Westerterp-Plantenga MS. Gluconeogenesis and protein-induced satiety. *Br J Nutr.* (2012) 107:595–600. doi: 10.1017/S0007114511003254

144. Crowder MC. *The Effect of Breakfast Protein Source on Postprandial Hunger and Glucose Response In Normal Weight and Overweight Young Women.* (2015). doi: 10.1096/fasebj.29.1_supplement.599.3

145. Pombo-Rodrigues S, Calame W, Re R. The effects of consuming eggs for lunch on satiety and subsequent food intake. *Int J Food Sci Nutr*. (2011) 62:593-99. doi: 10.3109/09637486.2011.566212

146. Borzoei S, Neovius M, Barkeling B, Teixeira-Pinto A, Rössner S, A. comparison of effects of fish and beef protein on satiety in normal weight men. *Eur J Clin Nutr.* (2006) 60:897–902. doi: 10.1038/sj.ejcn.1602397

147. Lang V, Bellisle F, Alamowitch C, Craplet C, Bornet F, Slama G, et al. Varying the protein source in mixed meal modifies glucose, insulin and glucagon kinetics in healthy men, has weak effects on subjective satiety and fails to affect food intake. *Eur J Clin Nutr.* (1999) 53:959–65. doi: 10.1038/sj.ejcn.1600881

148. Drummen M, Tischmann L, Gatta-Cherifi B, Adam T, Westerterp-Plantenga M. Dietary protein and energy balance in relation to obesity and co-morbidities. *Front Endocrinol.* (2018) 443. doi: 10.3389/fendo.2018.00443

149. Anderson GH, Moore SE. Dietary proteins in the regulation of food intake and body weight in humans. *J Nutr.* (2004) 134:974S−9S. doi: 10.1093/jn/134.4.974S

150. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature.* (1997) 387:903–8. doi: 10.1038/43185

151. Hansen HS. Role of anorectic N-acylethanolamines in intestinal physiology and satiety control with respect to dietary fat. *Pharmacol Res.* (2014) 86:18–25. doi: 10.1016/j.phrs.2014.03.006

152. Little TJ, Feinle-Bisset C. Effects of dietary fat on appetite and energy intake in health and obesity—oral and gastrointestinal sensory contributions. *Physiol Behav.* (2011) 104:613–20. doi: 10.1016/j.physbeh.2011.04.038

153. Romano A, Azari EK, Tempesta B, Mansouri A, Di Bonaventura MM, Ramachandran D, et al. High dietary fat intake influences the activation of specific hindbrain and hypothalamic nuclei by the satiety factor oleoylethanolamide. *Physiol Behav.* (2014) 136:55–62. doi: 10.1016/j.physbeh.2014.04.039

154. Welch I, Sepple C, Read N. Comparisons of the effect of infusion of lipid into the jejunum and ileum on eating behaviour and satiety in man. *Gut.* (1988) 29:306–11. doi: 10.1136/gut.29.3.306

155. Kozimor A, Chang H, Cooper JA. Effects of dietary fatty acid composition from a high fat meal on satiety. *Appetite.* (2013) 69:39–45. doi: 10.1016/j.appet.2013.05.006

156. Van Wymelbeke V, Louis-Sylvestre J, Fantino M. Substrate oxidation and control of food intake in men after a fat-substitute meal compared with meals supplemented with an isoenergetic load of carbohydrate, long-chain triacylglycerols, or medium-chain triacylglycerols. *Am J Clin Nutr.* (2001) 74:620–30. doi: 10.1093/ajcn/74.5.620

157. Himaya A, Fantino M, Antoine J-M, Brondel L, Louis-Sylvestre J. Satiety power of dietary fat: a new appraisal. *Am J Clin Nutr.* (1997) 65:1410–8. doi: 10.1093/ajcn/65.5.1410

158. Maher T, Clegg ME. Dietary lipids with potential to affect satiety: Mechanisms and evidence. *Crit Rev Food Sci Nutr.* (2019) 59:1619–44. doi: 10.1080/10408398.2017.1423277

159. Sandhu KS, El Samahi MM, Mena I, Dooley CP, Valenzuela JE. Effect of pectin on gastric emptying and gastroduodenal motility in normal subjects. *Gastroenterology.* (1987) 92:486–92. doi: 10.1016/0016-5085(87)90146-6

160. Sanaka M, Yamamoto T, Anjiki H, Nagasawa K, Kuyama Y. Effects of agar and pectin on gastric emptying and post-prandial glycaemic profiles in healthy human volunteers. *Clin Exp Pharmacol Physiol.* (2007) 34:1151–5. doi: 10.1111/j.1440-1681.2007.04706.x

161. Wilmshurst P, Crawley J. The measurement of gastric transit time in obese subjects using 24Na and the effects of energy content and guar gum on gastric emptying and satiety. *Br J Nutr.* (1980) 44:1–6. doi: 10.1079/BJN19800003

162. Yu K, Ke M-Y, Li W-H, Zhang S-Q, Fang X-C. The impact of soluble dietary fibre on gastric emptying, postprandial blood glucose and insulin in patients with type 2 diabetes. *Asia Pac J Clin Nutr.* (2014) 23:210–8.

163. Georg MG, Kristensen M, Belza A, Knudsen JC, Astrup A. Acute effect of alginate-based preload on satiety feelings, energy intake, and gastric emptying rate in healthy subjects. *Obesity.* (2012) 20:1851–8. doi: 10.1038/oby.2011.232

164. Hoad CL, Rayment P, Spiller RC, Marciani L, Alonso BDC, Traynor C, et al. In vivo imaging of intragastric gelation and its effect on satiety in humans. *J Nutr.* (2004) 134:2293–300. doi: 10.1093/jn/134.9.2293

165. Odunsi ST, Vázquez-Roque MI, Camilleri M, Papathanasopoulos A, Clark MM, Wodrich L, et al. Effect of alginate on satiation, appetite, gastric function, and selected gut satiety hormones in overweight and obesity. *Obesity.* (2010) 18:1579–84. doi: 10.1038/oby.2009.421

166. Wanders AJ, Jonathan MC, Van Den Borne JJ, Mars M, Schols HA, Feskens EJ, et al. The effects of bulking, viscous and gel-forming dietary fibres on satiation. *Br J Nutr.* (2013) 109:1330–7. doi: 10.1017/S0007114512003145

167. Hervik AK, Svihus B. The role of fiber in energy balance. *J Nutr Metab.* (2019) 2019:1–11. doi: 10.1155/2019/4983657

168. García-Carrizo F, Picó C, Rodríguez AM, Palou A. High-esterified pectin reverses metabolic malprogramming, improving sensitivity to adipostatic/adipokine hormones. *J Agric Food Chem.* (2019) 67:3633–42. doi: 10.1021/acs.jafc.9b00296

169. Kim M. High-methoxyl pectin has greater enhancing effect on glucose uptake in intestinal perfused rats. *Nutrition.* (2005) 21:372–7. doi: 10.1016/j.nut.2004.07.006

170. Palou M, Sánchez J, García-Carrizo F, Palou A, Picó C. Pectin supplementation in rats mitigates age-related impairment in insulin and leptin sensitivity independently of reducing food intake. *Mol Nutr Food Res.* (2015) 59:2022–33. doi: 10.1002/mnfr.201500292

171. Dongowski G, Lorenz A, Proll JR. The degree of methylation influences the degradation of pectin in the intestinal tract of rats and *in vitro*. *J Nutr.* (2002) 132:1935–44. doi: 10.1093/jn/132.7.1935

172. Hillman ET, Lu H, Yao T, Nakatsu CH. Microbial ecology along the gastrointestinal tract. *Microbes Environ.* (2017) ME17017. doi: 10.1264/jsme2.ME17017

173. Sánchez D, Muguerza B, Moulay L, Hernández R, Miguel M, Aleixandre A. Highly methoxylated pectin improves insulin resistance and other cardiometabolic risk factors in Zucker fatty rats. *J Agric Food Chem.* (2008) 56:3574–81. doi: 10.1021/jf703598j

174. Pino JL, Mujica V, Arredondo M. Effect of dietary supplementation with oat β-glucan for 3 months in subjects with type 2 diabetes: a randomized, double-blind, controlled clinical trial. *J Funct Foods.* (2021) 77:104311. doi: 10.1016/j.jff.2020.104311

175. Adam CL, Williams PA, Dalby MJ, Garden K, Thomson LM, Richardson AJ, et al. Different types of soluble fermentable dietary fibre decrease food intake, body weight gain and adiposity in young adult male rats. *Nutr Metab.* (2014) 11:1–12. doi: 10.1186/1743-7075-11-36

176. Osilesi O, Trout DL, Glover EE, Harper SM, Koh ET, Behall KM. (Use of xanthan gum in dietary management of diabetes mellitus. *Am J Clin Nutr.* (1985) 42:597–603. doi: 10.1093/ajcn/42.4.597

177. Zurakowski AR, Zahorska-Markiewicz B, Olszanecka-Glinianowicz M, Mucha Z. The effect of xantham gum on satiety status of obese patients after test meal. *Wia Lek.* (2005) 58:303–6.

178. Espert M, Salvador A, Sanz T. Rheological and microstructural behaviour of xanthan gum and xanthan gum-Tween 80 emulsions during in vitro digestion. *Food Hydrocoll.* (2019) 95:454–61. doi: 10.1016/j.foodhyd.2019.05.004

179. Clark MJ, Slavin JL. The effect of fiber on satiety and food intake: a systematic review. *J Am Coll Nutr.* (2013) 32:200–11. doi: 10.1080/07315724.2013.791194

180. Roe LS, Meengs JS, Rolls BJ. Salad and satiety. The effect of timing of salad consumption on meal energy intake. *Appetite.* (2012) 58:242–8. doi: 10.1016/j.appet.2011.10.003

181. Carter BE, Drewnowski A. Beverages containing soluble fiber, caffeine, and green tea catechins suppress hunger and lead to less energy consumption at the next meal. *Appetite.* (2012) 59:755–61. doi: 10.1016/j.appet.2012.08.015

182. Reinbach HC, Smeets A, Martinussen T, Møller P, Westerterp-Plantenga M. Effects of capsaicin, green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance. *Clin Nutr.* (2009) 28:260–5. doi: 10.1016/j.clnu.2009.01.010

183. Van Avesaat M, Troost FJ, Westerterp-Plantenga MS, Helyes Z, Le Roux CW, Dekker J, et al. Capsaicin-induced satiety is associated with gastrointestinal distress but not with the release of satiety hormones, 2. *Am J Clin Nutr.* (2016) 103:305–13. doi: 10.3945/ajcn.115.123414

184. Hunter DC, Jones VS, Hedderley DI, Jaeger SR. The influence of claims of appetite control benefits in those trying to lose or maintain weight: The role of claim believability and attitudes to functional foods. *Food Res Int.* (2019) 119:715–24. doi: 10.1016/j.foodres.2018.10.051

185. Blundell J. Making claims: functional foods for managing appetite and weight. *Nat Rev Endocrinol.* (2010) 6:53–6. doi: 10.1038/nrendo.2009.224

186. De Boer A, Urlings MJ, Bast A. Active ingredients leading in health claims on functional foods. *J Funct Foods.* (2016) 20:587–93. doi: 10.1016/j.jff.2015.11.025

187. López-Nicolás R, Marzorati M, Scarabottolo L, Halford JC, Johnstone AM, Frontela-Saseta C, et al. Satiety innovations: food products to assist consumers with weight loss, evidence on the role of satiety in healthy eating: overview and *in vitro* approximation. *Curr Obes Rep.* (2016) 5:97–105. doi: 10.1007/s13679-016-0196-9

188. Esmaeili M, Ajami M, Barati M, Javanmardi F, Houshiarrad A, Mousavi Khaneghah A. The significance and potential of functional food ingredients for control appetite and food intake. *Food Sci Nutr.* (2022) 10:1602–12. doi: 10.1002/fsn3.2783

189. Rebello CJ, Johnson WD, Martin C, Johnson J, O'Shea M, Chu Y. Effect of two oat-based cereals on subjective ratings of appetite. *Curr Top Nutraceutical Res.* (2018) 16:113.

190. Isaksson H, Fredriksson H, Andersson R, Olsson J, Åman P. Effect of rye bread breakfasts on subjective hunger and satiety: a randomized controlled trial. *Nutr J.* (2009) 8:39. doi: 10.1186/1475-2891-8-39

191. Trinidad TP, Tuaño APP, Juliano BO. Short-term satiety of cooked Philippine rices of varying apparent amylose content and glycemic index. *Philipp Agric Sci.* (2013) 6:179–86.

192. Costabile G, Griffo E, Cipriano P, Vetrani C, Vitale M, Mamone G, et al. Subjective satiety and plasma PYY concentration after wholemeal pasta. *Appetite.* (2018) 125:172–81. doi: 10.1016/j.appet.2018.02.004

193. Pai S, Ghugre P, Udipi S. Satiety from rice-based, wheat-based and rice–pulse combination preparations. *Appetite.* (2005) 44:263–71. doi: 10.1016/j.appet.2005.01.004

194. Kristensen M, Jensen MG, Riboldi G, Petronio M, Bügel S, Toubro S, et al. Wholegrain vs. refined wheat bread and pasta Effect on postprandial glycemia, appetite, and subsequent ad libitum energy intake in young healthy adults. *Appetite.* (2010) 54:163–9. doi: 10.1016/j.appet.2009.10.003

195. Giuberti G, Albertini E, Miggiano GA. Dall'Asta M, Rossi F. Effect of biscuits formulated with high-amylose maize flour on satiety-related sensations and food intake. *Int J Food Sci Nutr.* (2021) 72:1138–45. doi: 10.1080/09637486.2021.1911961

196. Zijlstra N, Mars M, Stafleu A, De Graaf C. The effect of texture differences on satiation in 3 pairs of solid foods. *Appetite.* (2010) 55:490–7. doi: 10.1016/j.appet.2010.08.014

197. Charlton KE, Tapsell LC, Batterham MJ, Thorne R, O'Shea J, Zhang Q, et al. Pork, beef and chicken have similar effects on acute satiety and hormonal markers of appetite. *Appetite.* (2011) 56:1–8. doi: 10.1016/j.appet.2010.10.013

198. Crowe W, McLaughlin CM, Allsopp PJ, Slevin MM, Harnedy PA, Cassidy Y, et al. The effect of boarfish protein hydrolysate on postprandial glycaemic response and satiety in healthy adults.In: *Proceedings of the Nutrition Society.* (2018) 77. doi: 10.1017/S002966511800109X

199. Bonnema AL, Altschwager D, Thomas W, Slavin JL. The effects of a beef-based meal compared to a calorie matched bean-based meal on appetite and food intake. *J Food Sci.* (2015) 80:H2088–93. doi: 10.1111/1750-3841.12991

200. Maljaars J, Romeyn EA, Haddeman E, Peters HP, Masclee AA. Effect of fat saturation on satiety, hormone release, and food intake. *Am J Clin Nutr.* (2009) 89:1019–24. doi: 10.3945/ajcn.2008.27335

201. Kinsella R, Maher T, Clegg M. Coconut oil has less satiating properties than medium chain triglyceride oil. *Physiol Behav.* (2017) 179:422–6. doi: 10.1016/j.physbeh.2017.07.007

202. Alfenas RC, Mattes RD. Effect of fat sources on satiety. *Obes Res.* (2003) 11:183–7. doi: 10.1038/oby.2003.29

203. Smith-Ryan AE, Hirsch KR, Blue MN, Mock MG, Trexler ET. High-fat breakfast meal replacement in overweight and obesity: implications on body composition, metabolic markers, and satiety. *Nutrients.* (2019) 11:865. doi: 10.3390/nu11040865

204. Dong H, Sargent LJ, Chatzidiakou Y, Saunders C, Harkness L, Bordenave N, et al. Orange pomace fibre increases a composite scoring of subjective ratings of hunger and fullness in healthy adults. *Appetite.* (2016) 107:478–85. doi: 10.1016/j.appet.2016.08.118

205. Zhu L, Huang Y, Edirisinghe I, Park E, Burton-Freeman B. Using the avocado to test the satiety effects of a fat-fiber combination in place of carbohydrate energy in a breakfast meal in overweight and obese men and women: a randomized clinical trial. *Nutrients.* (2019) 11:952. doi: 10.3390/nu11050952

206. Magrane EJ. *The Effects of Blueberry Consumption on Satiety and Glycemic Control.* University of Maine. (2009).

207. Lum T, Connolly M, Marx A, Beidler J, Hooshmand S, Kern M, et al. Effects of fresh watermelon consumption on the acute satiety response and cardiometabolic risk factors in overweight and obese adults. *Nutrients.* (2019) 11:595. doi: 10.3390/nu11030595

208. Pinneo S, O'Mealy C, Rosas Jr M, Tsang M, Liu C, Kern M. Fresh mango consumption promotes greater satiety and improves postprandial glucose and insulin responses in healthy overweight and obese adults. *J Med Food.* (2022) 25:381–8. doi: 10.1089/jmf.2021.0063

209. Kaplan RJ, Greenwood CE. Influence of dietary carbohydrates and glycaemic response on subjective appetite and food intake in healthy elderly persons. *Int J Food Sci Nutr.* (2002) 53:305–16. doi: 10.1080/09637480220138160

210. Andersen SS, Heller JM, Hansen TT, Raben A. Comparison of low glycaemic index and high glycaemic index potatoes in relation to satiety: a single-blinded, randomised crossover study in humans. *Nutrients.* (2018) 10:1726. doi: 10.3390/nu10111726

211. Mathern JR, Raatz SK, Thomas W, Slavin JL. Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. *Phytother Res.* (2009) 23:1543–8. doi: 10.1002/ptr.2795

212. Westerterp-Plantenga M, Janssens PL. Red pepper can enhance energy metabolism and satiety. *Nutr Today.* (2014) 49:S6–7. doi: 10.1097/01.NT.0000453845.91592.11

213. Moorhead SA, Welch RW, Barbara M, Livingstone E, Mccourt M, Burns AA, et al. The effects of the fibre content and physical structure of carrots on satiety and subsequent intakes when eaten as part of a mixed meal. *Br J Nutr.* (2006) 96:587–95. doi: 10.1079/BJN20061790

214. Morell P, Piqueras-Fiszman B, Hernando I, Fiszman S. How is an ideal satiating yogurt described? A case study with added-protein yogurts. *Food Res Int.* (2015) 78:141–7. doi: 10.1016/j.foodres.2015.10.024

215. Onvani S, Haghighatdoost F, Surkan PJ, Azadbakht L. Dairy products, satiety and food intake: a meta-analysis of clinical trials. *Clin Nutr.* (2017) 36:389–98. doi: 10.1016/j.clnu.2016.01.017

216. Rubio-Martín E, García-Escobar E, Ruiz De Adana M-S, Lima-Rubio F, Peláez L, Caracuel A-M, et al. Comparison of the effects of goat dairy and cow dairy based breakfasts on satiety, appetite hormones, and metabolic profile. *Nutrients.* (2017) 9:877. doi: 10.3390/nu9080877

217. Kung B, Anderson G, Paré S, Tucker A, Vien S, Wright A, et al. Effect of milk protein intake and casein-to-whey ratio in breakfast meals on postprandial glucose, satiety ratings, and subsequent meal intake. *J Dairy Sci.* (2018) 101:8688–701. doi: 10.3168/jds.2018-14419

218. Schellekens H, Nongonierma AB, Clarke G, Van Oeffelen WE, Fitzgerald RJ, Dinan TG, et al. Milk protein-derived peptides induce 5-HT2C-mediated satiety *in vivo*. *Int Dairy J.* (2014) 38:55–64. doi: 10.1016/j.idairyj.2014.04.004

219. Mather K, Boachie R, Anini Y, Panahi S, Anderson GH, Luhovyy BL. Effects of cultured dairy and nondairy products added to breakfast cereals on blood glucose control, satiation, satiety, and short-term food intake in young women. *Appl Physiol Nutr Metab.* (2020) 45:1118–26. doi: 10.1139/apnm-2019-0772

220. Marsset-Baglieri A, Fromentin G, Airinei G, Pedersen C, Léonil J, Piedcoq J, et al. Milk protein fractions moderately extend the duration of satiety compared with carbohydrates independently of their digestive kinetics in overweight subjects. *Br J Nutr.* (2014) 112:557–64. doi: 10.1017/S0007114514001470

221. Mehrabani S, Safavi SM, Mehrabani S, Asemi M, Feizi A, Bellissimo N, et al. Effects of low-fat milk consumption at breakfast on satiety and short-term energy intake in 10-to 12-year-old obese boys. *Eur J Nutr.* (2016) 55:1389–96. doi: 10.1007/s00394-015-0956-4

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Validation and results of a novel survey assessing decisional balance for a whole food plant-based diet among US adults

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Importance: Consuming a whole food plant-based diet (WFPBD) is a promising, low-risk strategy for reducing risk of prevalent chronic disease and certain cancers, with synergistic benefits for climate and environment. However, few US adults report consuming a WFPBD. Understanding the reasons for this inconsistency is important for developing and implementing interventions for promoting a WFPBD. However, no research to elucidate decisional balance driving current consumption patterns in the US exists.

Objective: This research aims to validate an online survey to assess decisional balance for the consumption of a WFPBD, describe attitudes and beliefs toward adopting a WFPBD, and evaluate socio-demographic differences in decisional balance for consuming a WFPBD among a convenience sample of US adults.

Design: Online cross-sectional data collection followed by confirmatory factor analysis (CFA), validation of internal consistency, and examination of invariance across socio-demographic variables. Sensitivity analysis of full vs. truncated survey to predict self-reported dietary patterns and consumption behaviors were evaluated. Results of the survey and significant differences by socio-demographics were assessed.

Setting: Online survey based on previous research, created *via* Qualtrics, and administered through MTurk.

Participants: A total of 412 US adults, majority female (66%), White (75%), 30–60 years old (54%), $\geq$ Bachelor's degree (85%), and earning $\geq$ \$45K (68%).

Main outcomes and measures: Factor loadings, covariance of survey items, associations with self-reported dietary pattern and consumption measures, and differences in pros, cons, and decisional balance across socio-demographic variables.

Results: CFA reduced the survey from 49 to 12 items and demonstrated invariance across socio-demographic variables. Pros and cons varied inversely and significantly (cov = −0.59), as expected. Cronbach's α 's for subscales in the final, reduced model were high (>0.80). Pros, cons, and decisional balance in both the full and the reduced model were significantly ($p < 0.05$) associated with self-reported dietary pattern and consumption.

Conclusion and relevance: Our analyses indicate the WFPBD Survey is a parsimonious and psychometrically sound instrument for evaluation of decisional balance to consume a WFPBD diet among our sample of US adults. These results may be instrumental for development and deployment of interventions intended to promote consumption of a WFPBD in the US.

KEYWORDS

whole food plant-based diet, nutrition behavior, decisional balance, confirmatory factor analysis, psychometric properties, consumption pattern

Background

Diet is a critical component of human health (1–3) and is strongly implicated in the incidence of several chronic diseases, including cardiovascular disease (CVD), coronary artery disease (CAD), overweight and obesity, and type 2 diabetes (T2D), as well as certain cancers (4–10). Conversely, a whole food plant-based diet (WFPBD), defined as a pattern of consumption emphasizing minimally processed fruits, vegetables, legumes, nuts, seeds, and whole grains while minimizing meat, eggs, and dairy (11), has been shown to have numerous positive effects on overall human health (12–17), reducing risk for metabolic syndrome (18), obesity (19, 20), CVD (21, 22), T2D (23–25), and several diet-related cancers (26, 27). Additionally, increasing consumption of plant-based foods and decreasing consumption of meat has synergistic environmental impacts for reducing greenhouse gas emissions, decreasing land and water use, and protecting biodiversity (28–32). Widespread adoption and normalization of a WFPBD may simultaneously reduce the incidence of chronic disease and cancer while improving the environmental footprint of diet (31, 33).

Despite these potentially substantial benefits to human health and the environment, the number of people in the U.S. who report consuming a WFPBD remains very low (∼5%) relative to those who consume other, more traditional Western diets (34). In fact, less than 15% of adults in the US meet recommended levels of fruit and vegetable (FV) consumption (35). In 2017, per capita meat consumption in the US was 217 pounds, or about 3 times the global average (36), despite the increasing evidence in favor of WFPBD in promoting human and environmental health. Understanding the reasons for this disconnect is the first step in designing policies and interventions that may be effective in increasing uptake of a WFPBD in the US.

Research in Australia, Scotland, Portugal, and the Netherlands has revealed that attitudes and beliefs toward a WFPBD exhibit variation within and among sociodemographic and ethnic groups, and these differences vary among countries and over time (11, 30, 37, 38). For example, consumers in Europe reported significant differences in attitudes toward beef and pork, and these differences varied significantly across countries (39). In the US, the ADAPT study used a single multiple-choice question to find significantly more plant-based diet followers (including vegans, vegetarians, and pescatarians) compared to omnivores identified helping the environment and animal welfare as their top motivations (40). Other research in the US has posited philosophical reasons for consuming a plant-based diet, such as motivations, aversions, and constraints (41). However, there is a paucity of validated tools for assessing psychosocial constructs relevant for understanding current decisional balance driving consumption of a WFPBD in the US. Thus, we propose a pragmatic and theory-driven approach to developing tools and evidence to guide the design and implementation of interventions and policies intended to increase consumption of a WFPBD. This research will address this goal by achieving the following aims:

(1) Validate an online survey tool to assess attitudes and beliefs related to the consumption of a WFPBD among a convenience sample of 412 adults in the US,

(2) Describe attitudes and beliefs toward adopting a WFPBD among a sample of US adults, and

(3) Evaluate socio-demographic differences in attitudes and beliefs toward WFPBD among a sample of US adults.

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Materials and methods

Survey development

The WFPBD survey was organized around the theory of decisional balance (pros vs. cons), posited by Janis and Mann (42), elaborated by Prochaska in the Transtheoretical Model (43–45), and found to be successful in predicting dietary behaviors (43–45). Questions were adapted from previous surveys administered in US and non-US populations (11, 37, 38, 46–48). Forty-nine survey questions adapted from Lea, Crawford, and Wolsey's survey of 415 Australian adults were formatted as 5-item Likert scales, with responses: strongly disagree/disagree/not sure/agree/strongly agree. Items were selected to represent a two-factor model (pros and cons) with 23 items assessing cons of a WFPBD and 26 items assessing pros. To provide a theoretical and organizational framework, these 49 items were grouped to correspond to seven psychosocial constructs from Social Cognitive Theory (SCT) for each factor: social support, instrumental support, self-efficacy, perceived health impacts, taste preferences, knowledge, and attitudes toward animals. SCT constructs have been shown to be significantly associated with dietary behavior change in a variety of populations (49, 50). In addition, the survey contained demographic questions (5 items), dietary questions (6 items) adapted from the American Heart Association's Rapid Diet Screener Tool for Clinicians (51) and 3 questions assessing motivational willingness to reduce meat consumption sourced from a previously validated survey (37), for a total of 77 questions (**Supplementary Appendix A**). The survey was administered using Qualtrics (Qualtrics©, Provo, UT, US) between August 30 and 31, 2021.

Participant screening and recruitment

Participants were screened and recruited *via* Amazon's Mechanical Turk (MTurk), an online platform that has been shown to offer a larger and more representative sample pool than traditional forms of convenience sampling can achieve (52–55). An initial screening survey was administered based on the study's inclusion criteria: 18 years or older, fluent in English, and a resident of the US. Those who met the inclusion criteria were then directed to the consent document and asked to participate. If consent was given, the participant was then presented with the survey, and upon completion (as validated by a unique completion code generated once the survey was completed), the participant received $10 in compensation *via* the MTurk platform. Approval for this study was granted by the Institutional Review Board of Northwestern University (IRB ID: STU0054672).

Analyses

Confirmatory Factor Analysis (CFA) was employed to validate the survey, and to determine if a more parsimonious tool would be as effective in capturing subject's attitudes and beliefs toward a WFPBD, thus reducing survey burden. To achieve this, a CFA model was fitted using pros and cons of a WFPBD as latent variables that explained variance in relevant survey items. Because latent variable data were ordinal and not multivariate normal, ordinal logistic regression within a generalized structural equation model was used. For each factor (pros and cons), self-efficacy was used as the referent construct. Following the first CFA with all 49 items, those items with factor loadings < 0.80 were removed from the model. Then, modification indices were examined and item pairs with $\chi^2 > 3.84$ were assessed, and the variable with the lower factor loading was removed from the model. The same process was followed a second time to arrive at the final model. Cronbach's α 's were used to assess internal consistency. Model fit statistics were generated (AIC, BIC), and Average Variance Explained (AVE) and Discriminant

TABLE 1 Demographic characteristics of the WFPBD survey sample.

	Total sample (N = 412)
Sex	***n*** **(%)**
Male	272 (66.02)
Female	139 (33.74)
Non-binary	1 (0.24)
AGE	
< 30 years	178 (43.10)
Between 30 and 60 years	222 (53.75)
> 60 years	13 (3.15)
Ethnicity	
African American/Black	48 (11.65)
Latino	25 (6.07)
White	309 (75.00)
Asian	25 (6.07)
Other	5 (1.21)
Education	
Less than Bachelor's	63 (15.29)
Bachelor's or higher	349 (84.71)
INCOME	
< $45,000/year	133 (32.28)
≥ $45,000/year	279 (67.72)
Diet pattern	
No special diet	113 (27.43)
Vegan	59 (14.32)
Vegetarian	121 (29.37)
Pesco-vegetarian	59 (14.32)
Flexitarian	60 (14.56)

Value (DV) calculated to provide measures of convergent and divergent validity, respectively. Invariance testing of the final model across sex, age, race/ethnicity, education, and income was performed to assess whether factor loadings varied significantly by demographic category.

Following CFA, pros and cons were evaluated following procedures originated by Janis and Mann (42) and utilized across a number of health behaviors (smoking, physical activity, diet) over several decades (43, 44, 56–59). First, pros and cons were calculated as the summed average grouped by factor (i.e., pros and cons), and decisional balance was calculated as the difference between pros and cons (pros minus cons). In addition, a categorical variable for each construct was generated, with 0 = strongly disagree/ disagree/ not sure and 1 = agree/ strongly agree. We compared the reduced WFPBD Survey to the original, using linear regression to assess whether the pros, cons, and decisional balance extracted from the shorter survey (independent variables) were able to predict self-reported consumption behaviors (i.e., self-reported daily servings of fruits and vegetables, and of meats, eggs, and dairy) (51) (dependent variables) at least as well as the original, adjusted for relevant demographic variables (age, income, education, and race/ethnicity). Finally, pros, cons, and decisional balance using the reduced, final model of the WFPBD Survey were evaluated, and significant differences, alone and across relevant demographic variables, were assessed *via* chi-square tests. Significance was assessed as a p-value < 0.05. All analyses were conducted using STATA version 16.1 (StataCorp (60)).

Results

Our sample was primarily White (75%), 30–60 years old (54%), had a Bachelor's degree or higher (85%), and earned $45K or more (68%). Close to one third of participants self-reported consuming no special diet and one-third self-reported consuming a vegetarian diet (no meat, includes dairy), with remainder evenly split (∼14%) between vegan (no animal products), pesco-vegetarian (vegetarian plus fish), and flexitarian (mostly vegetarian with minimal animal products) dietary patterns (**Table 1**).

Survey validation

Following initial CFA, the model was iteratively reduced based on cut-points of 0.80 for factor loadings and 3.84 for modification indices, which yielded a final model with 6 items each for pros and cons of a WFPBD. In the full model (Model 1), highest factor loading among WFPBD pros was 0.97 for Q67 ("It helps me build or maintain muscle.") and lowest was 0.81 for Q64 ("Generally, eating a WFPBD gives me a better quality of life."), while for cons items the highest factor loading was 1.46 for Q32 ("I wouldn't get enough energy or strength."), and lowest was 1.25 for Q36 ("I don't know what to eat instead of meat."). In all models, all factor loadings were significant. Cronbach's α 's for both pros and cons subscales in the final, reduced model (Model 2) were high (> 0.80) (**Table 2**). Covariance between pros and cons of a WFPBD was –0.59 ($p < 0.05$), so that these latent variables are significantly and inversely related, as hypothesized (**Figure 1**).

Model fit was assessed using AIC and BIC. With each iteration of the model, AIC and BIC decreased, indicating the model fit increased as factor loadings were optimized, cross loadings minimized, and items reduced (**Table 3**: Model Fit Statistics for CFA iterations of the WGPBD Survey). For the final model, convergent validity was explored using AVE and discriminant validity was assessed *via* the DV. A model is generally regarded as having acceptable convergent validity

TABLE 2 Factor loadings (SE), *p*-values, 95% CI's, and R-squared statistics for the final WFPBD following confirmatory factor analysis.

WFPBD survey item	Factor loading (SE)	*P*-value	95% CI	R^2
PROs subscale: Cronbach's α = 0.88				
Q47. My friends and family think eating a WFPBD is best.	0.90 (0.11)	<0.001	0.69, 1.12	0.52
Q53. I am confident I can eat a plant-based diet forever.	1.00 (Constrained)	NA	NA	0.58
Q63. A WFPBD is part of being fit.	0.86 (0.12)	<0.001	0.63, 1.09	0.55
Q64. Generally, eating a WFPBD helps me have a better quality of life.	0.81 (0.11)	<0.001	0.60, 1.02	0.56
Q65. I have plenty of energy on a WFPBD.	0.86 (0.12)	<0.001	0.63, 1.09	0.55
Q67. It helps me build or maintain muscle.	0.97 (0.12)	<0.001	0.74, 1.20	0.55
CONs subscale: Cronbach's α = 0.86				
Q26. I do not feel confident that I have enough willpower to eat a plant-based diet.	1.00 (Constrained)	NA	NA	0.41
Q30. There is not enough iron in plant foods.	1.42 (0.19)	<0.001	1.05, 1.78	0.52
Q32. I wouldn't get enough energy or strength.	1.46 (0.20)	<0.001	1.06, 1.85	0.57
Q35. I would lose muscle.	1.40 (0.19)	<0.001	1.03, 1.76	0.51
Q42. I don't know how to prepare plant-based meals.	1.38 (0.17)	<0.001	1.04, 1.72	0.54
Q43. I don't know what to eat instead of meat.	1.25 (0.16)	<0.001	0.94, 1.56	0.50

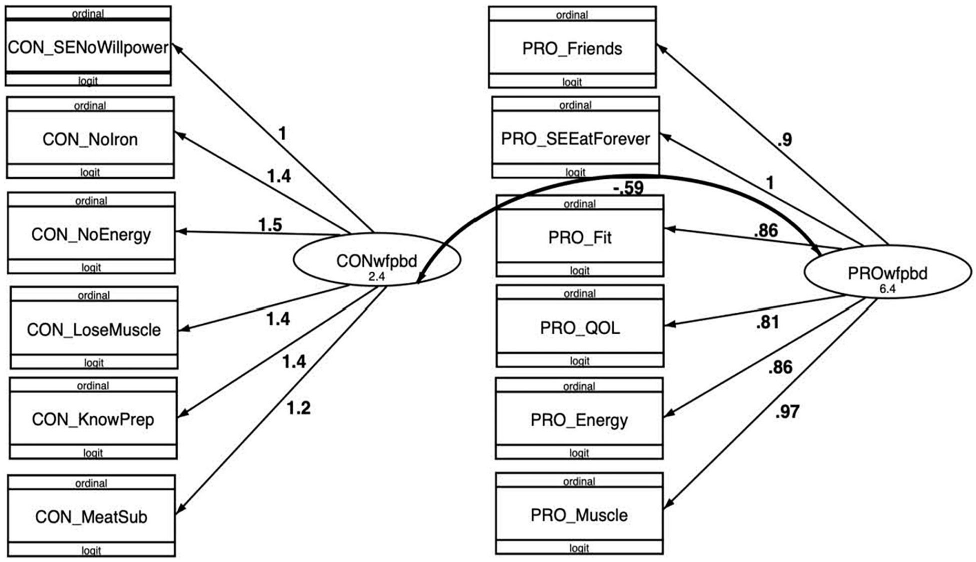

FIGURE 1
Final (12-item) generalized logit confirmatory factor analysis (CFA) model for WFPBD Survey, with factor loadings and covariances.

if the AVE is at least 0.50 and good convergent validity at an AVE above 0.70. Our final model resulted in an AVE of 0.61 for pros of a WFPBD and 0.57 for cons, such that the model has acceptable convergent validity. DV's for the latent variables were both above recommended cut point of 0.70 (pros DV = 0.90, cons DV = 0.85), allowing us to conclude that the final model also displayed good divergent validity (61). Invariance testing was performed to assess whether factor loadings varied significantly across relevant demographic characteristics: age, sex, race/ethnicity, education, and income. Across all variables, the final model was invariant, with no significant differences among factor loadings for either pros or cons, indicating that the survey was consistent in measuring pros and cons across age, sex, race/ethnicity, education, and income.

To compare the original WFPBD Survey (Model 1, 49 items) to the final version (Model 3, 12 items), we fit linear regressions to assess whether the pros, cons, and decisional balance extracted from the final survey (independent variables) were able to predict self-reported consumption behaviors (i.e., self-reported daily servings of fruits and vegetables, and of meats, eggs, and dairy) (dependent variables) at least as well as the original, adjusted for relevant demographic variables (age, sex, race/ethnicity, education, and income). A similar pattern was found for both surveys, with significant β 's for predicting fruit and vegetable consumption and meat, egg, and dairy consumption, with the notable exception of pros variables to predict fruit and vegetable consumption for both the original (Model 1) and the final reduced (Model 3) WFPBD Survey (**Table 4**).

TABLE 3 Model fit statistics for confirmatory factor analysis iterations of the Whole food Plant-Based Diet Survey.

	Model 1	Model 2	Model 3
Number of items	49	21	12
Log likelihood	−30,533.37	−23,924.99	−7935.58
AIC	61,678.73	48,321.92	16,033.15
BIC	62,909.16	49,270.95	16,358.36

Survey results

In the final model (Model 3), mean value for pros (3.76) was slightly higher than for cons (3.03), and both were significantly associated with dietary pattern ($p < 0.001$ for both). Logistic regressions revealed that the crude and adjusted models both show that decisional balance is a significant predictor ($p < 0.001$) of dietary pattern. In the adjusted model, compared to those who reported consuming "No special diet," a one-unit increase in decisional balance is associated with a 2.14 times increased likelihood of reporting a vegan dietary pattern (no animal products) and a 1.55 times increased likelihood of reporting a flexitarian diet (mostly vegetarian with small amounts of meat and fish) (62). Overall effect of education is statistically significant in predicting the relationship between decisional balance and diet pattern ($\chi^2 = 45.54$, $p < 0.001$). However, no significant interactions between education and decisional balance were found.

Evaluating the final model using χ^2 tests, significant differences ($p < 0.05$) for pros, cons, and decisional balance

TABLE 4 Pros, cons, and decisional balance (IVs) and self-reported consumption outcomes (fruit and vegetables, and meat, eggs, and dairy) (DVs) using logistic regression adjusted for age, sex, income, race/ethnicity, and education.

	Model 1 (49 items)		Model 3 (12 items)	
	β (SE)	*P*-value	β (SE)	*P*-value
Fruits and vegetables				
Pros	0.10 (0.14)	$p = 0.47$	0.57 (0.11)	$p = 0.60$
Cons	−0.71 (0.10)	$p < 0.001$	−0.56 (0.08)	$p < 0.001$
Decisional balance	−0.03 (0.01)	$p < 0.001$	0.38 (0.01)	$p < 0.001$
Meat, eggs, and dairy				
Pros	−0.85 (0.22)	$p < 0.001$	−0.63 (0.17)	$p < 0.001$
Cons	0.83 (0.17)	$p < 0.001$	0.62 (0.13)	$p < 0.001$
Decisional balance	0.02 (0.01)	$p < 0.001$	−0.53 (0.01)	$p < 0.001$

were found across demographic variables. For pros and decisional balance, significant differences by age, education, and income were found, while cons varied significantly only by race/ethnicity (**Figure 2**). For age, those less than 30 years old had higher pros, while those between 30 and 60 years had higher cons. Participants older than 60 were ambivalent, exhibiting low levels of both pros and cons. Education also varied similarly for pros and decisional balance, with those with at least a Bachelor's degree expressing higher agreement with pros and positive decisional balance. Also of note were the relatively higher cons and negative decisional balance for those with less than a Bachelor's degree. Income followed the same pattern, with higher pros for those earning $45K or higher, and higher cons for those making less than $45K.

Discussion

The CFA of the WFPBD Survey resulted in a more parsimonious version that exhibited high factor loadings and strong validity, reducing the number of items from 49 to 12. This smaller number of items reduces survey burden and improves the utility of the WFPBD survey for inclusion in other survey instruments. Invariance testing revealed no significant differences in factor loadings across demographic variables, suggesting that the final WFPBD Survey is valid across a variety of demographics. Cronbach's alphas were above the 0.70 cutoff to indicate good internal consistency, and below 0.90, indicating minimal redundancy among the questions (63). Pros and cons of a WFPBD covaried inversely, as expected (β = −0.69, p = 0.03), demonstrating that pros increase as cons decrease, and vice versa. Both convergent validity (as expressed by the AVE above 0.50 for both pros and cons) and divergent validity (DV > 0.50 for pros and cons) were within acceptable ranges, indicating that the final survey items measured latent constructs as hypothesized. Further analysis of the final model revealed that the theoretical framework of pros, cons, and decisional balance was associated, as expected, with self-reported dietary pattern, providing further evidence that the reduced WFPBD Survey was able to measure the latent variables as intended. Our comparison of the original vs. reduced WFPBD Survey for predicting self-reported consumption of fruit and vegetables and meat, eggs, and dairy provides evidence that the reduced survey can elucidate significant predictors of these dietary behavior targets. Thus, by several important metrics, the WFPBD Survey demonstrates stable and high factor loadings, good internal consistency, acceptable levels of convergent and discriminant validity, and factor invariance, providing evidence that the reduced survey successfully measured the intended constructs, namely the pros, cons, and decisional balance for consuming a WFPBD in a sample of US adults. Additionally, our findings that pros and cons successfully predicted self-reported dietary intakes suggests that these psychosocial constructs are important for driving dietary choices, and, thus, accessible targets for interventions that seek to encourage consumption of a WFPBD.

The WFPBD Survey was able to detect significant differences among our sample, with decisional balance of pros and cons tracking with dietary pattern, and revealing important and significant variations across income, education, and race/ethnicity. For example, cons were significantly higher for Hispanic and African American participants, suggesting that barriers to consuming a WFPBD may be most important in these populations, as compared to pros. Being less than 60 years old, making at least $45K annually, and having at least a Bachelor's degree were all significantly associated with higher pros and lower cons for a WFPBD. This aligns with other research, such as Lea et al.'s (11) survey assessing perceptions of the benefits and barriers to eating a plant-based diet among 415 Australian adults. In this study, significant differences in perceived benefits and barriers were detected by sex, age, and education, similar to our results (11). Other research has found consumer attitudes and behaviors vary by country and have changed over time, even within the same demographics and countries. For instance, research in both Finland (64, 65) and Europe (39) has discovered shifts in consumer concerns around consumption of meat, from a more safety-focused paradigm to one that prioritizes health implications. Similarly, Fresán et al. (66) found motivations for consuming a plant-based diet to vary between California (health, social norms, religion) and Copenhagen (animal welfare, health, environment) (66). While these studies provide some insight into the reasons consumers may or may not chose to consume a WFPBD, these heterogenous results are attributable to culturally specific and dynamic processes and cannot be assumed to translate to adults in the US currently. Research in the US has found important differences in motivations between plant-based consumers and omnivores (40, 41), but did not validate the surveys used in these studies. This highlights the literature

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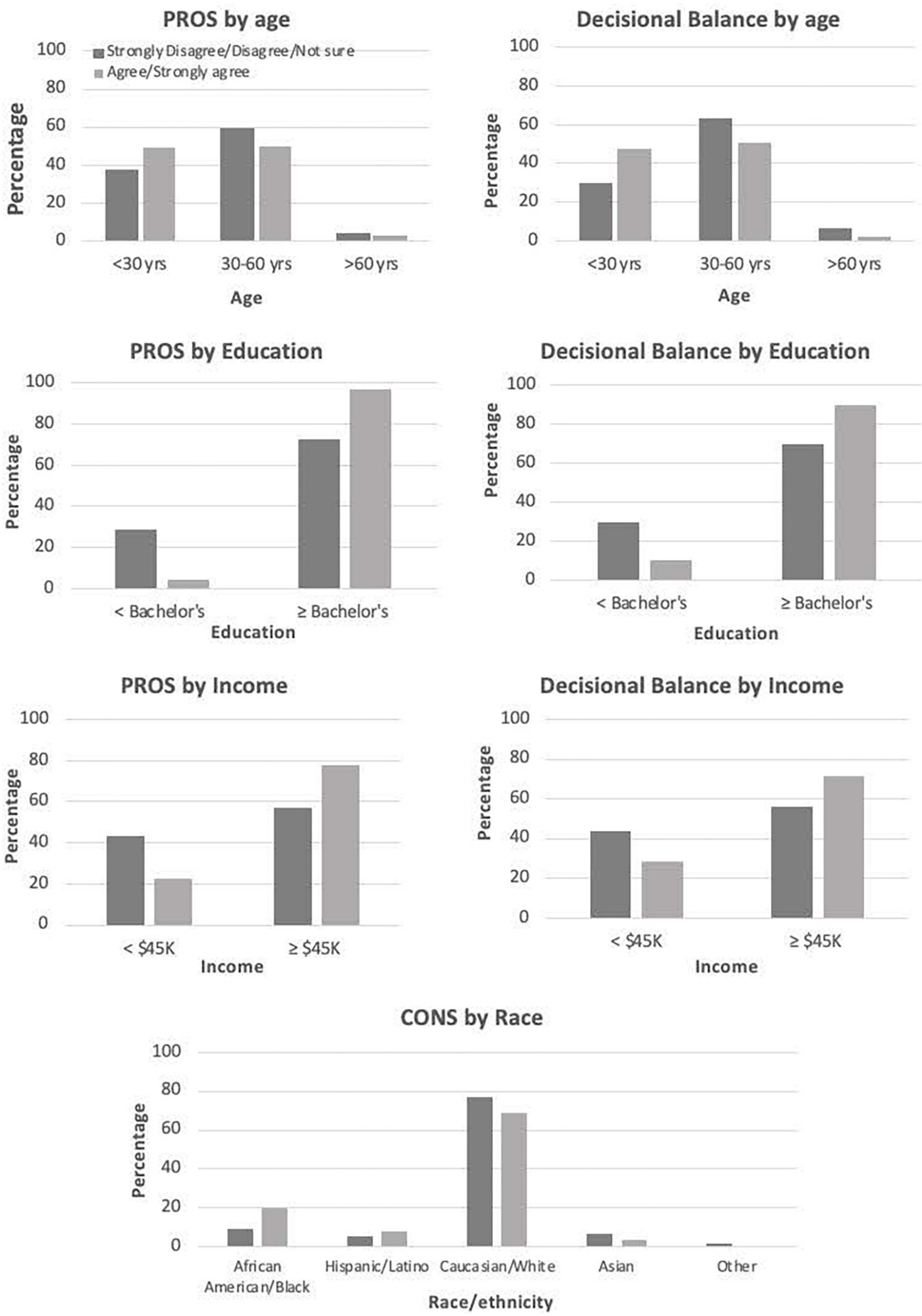

FIGURE 2
Significant ($p < 0.05$) differences in pros, cons, and decisional balance by demographic variables.

gap which this study aims to fill by providing a validated instrument suitable for US adults, as well as results from this convenience sample. These results may be instrumental for development and deployment of interventions intended to promote consumption of a WFPBD in US adults. In particular, the failure of pros to predict self-reported consumption of fruits and vegetables along with significant associations for decisional balance and cons, suggests that addressing cons in future interventions is the more effective approach to promoting consumption of a WFPBD. Additionally, our findings of significant differences in cons across race/ethnicity suggests that tailoring interventions with particular attention to racial/ethnic differences is important, especially for African American and Hispanic populations.

Limitations of this study include the lack of a test-retest reliability measure, since the survey was taken only once. In addition, all measures of both dietary pattern and consumption were self-reported, and therefore potentially subject to a number of biases, including imprecise recall and social desirability bias. Finally, this sample has a higher percentage of White participants (61.6% for the US vs. 75% for this study) and fewer Hispanic participants (18.7% for the US vs. 6.07% for this study) than the US in 2020 (67). Our sample was also more educated than average (84.71% for this study vs. 37.5% in the US with a Bachelor's degree or higher (68)) and more affluent (67.72% for this study vs. 53.74% in the US earning $45K or more (69)). Given the significant differences in pros, cons, and decisional balance we found across age, income, and education, the differences between this study's population and the US population are relevant for judging the generalizability of our results.

While our results elucidate several important and significant variations that may be useful for tailoring pro-WFPBD interventions to adult US participants by age, education, and income, further research is needed to assess whether these differences are consistent in other groups, such as Hispanic audiences, immigrant populations, or less affluent people. These differences were not detected in our relatively non-diverse sample. However, the validation measures we report here may be useful in achieving this goal by providing a psychometrically sound instrument with which to assess these psychosocial constructs in diverse populations. For example, a WFPBD has been shown to reduce levels of prostate-specific antigen in biopsy-confirmed indolent prostate cancer (70), which disproportionately affects African American men in US (71). The WFPBD Survey may be an important first step for understanding how to design and deploy an effective intervention to promote consumption of a WFPBD among African American men with indolent prostate cancer. The results from this study, as well as future uses of the WFPBD survey, provide evidence upon which we can build interventions to encourage a WFPBD for better health and environmental outcomes in the US.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Northwestern University (IRB ID: STU0054672). The patients/participants provided their written informed consent to participate in this study.

Author contributions

CJ designed the study, conducted primary data collection, performed data analysis, created tables, and served as primary author. FK assisted in writing the study proposal, created figures, and collaborated on writing the manuscript. FG assisted in writing the study proposal and collaborated on writing the manuscript. AP assisted in developing the survey and edited the manuscript. BS assisted in developing the study design, edited the manuscript, and provided funding. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.958611/full#supplementary-material

References

1. World Health Organization [WHO], *Healthy Diet*. Geneva: World Health Organization (2019).

2. Chen Y, Michalak M, Agellon LB. Focus: nutrition and food science: importance of nutrients and nutrient metabolism on human health. *Yale J Biol Med.* (2018) 91:95.

3. Tucker KL. The role of diet in chronic disease. In: Marriott B, Birt D, Stalling V, Yates A editors. *Present Knowledge in Nutrition*. Amsterdam: Elsevier (2020). p. 329–45. doi: 10.1016/B978-0-12-818460-8.00018-6

4. Bowman BA, Mokdad AH. Addressing nutrition and chronic disease: past, present, and future research directions. *Food Nutr Bull.* (2020) 41:3–7. doi: 10.1177/0379572119893904

5. Papadimitriou N, Markozannes G, Kanellopoulou A, Critselis E, Alhardan S, Karafousia V, et al. An umbrella review of the evidence associating diet and cancer risk at 11 anatomical sites. *Nat Commun.* (2021) 12:1–10. doi: 10.1038/s41467-021-24861-8

6. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5· 24 million UK adults. *Lancet.* (2014) 384:755–65. doi: 10.1016/S0140-6736(14)60892-8

7. Esposito K, Ciardiello F, Giugliano D. Unhealthy diets: a common soil for the association of metabolic syndrome and cancer. *Endocrine.* (2014) 46:39–42. doi: 10.1007/s12020-013-0151-4

8. Oyebode O, Gordon-Dseagu V, Walker A, Mindell JS. Fruit and vegetable consumption and all-cause, cancer and CVD mortality: analysis of health survey for England data. *J Epidemiol Community Health.* (2014) 68:856–62. doi: 10.1136/jech-2013-203500

9. Joshipura KJ, Hung H-C, Li TY, Hu FB, Rimm EB, Stampfer MJ, et al. Intakes of fruits, vegetables and carbohydrate and the risk of CVD. *Public Health Nutr.* (2009) 12:115–21. doi: 10.1017/S1368980008002036

10. Kim H, Caulfield LE, Garcia-Larsen V, Steffen LM, Coresh J, Rebholz CM. Plant-Based diets are associated with a lower risk of incident cardiovascular disease, cardiovascular disease mortality, and All-Cause mortality in a general population of Middle-Aged adults. *J Am Heart Assoc.* (2019) 8:e012865. doi: 10.1161/JAHA.119.012865

11. Lea EJ, Crawford D, Worsley A. Consumers' readiness to eat a plant-based diet. *Eur J Clin Nutr.* (2006) 60:342–51. doi: 10.1038/sj.ejcn.1602320

12. Medawar E, Huhn S, Villringer A, Veronica Witte A. The effects of plant-based diets on the body and the brain: a systematic review. *Transl Psychiatry.* (2019) 9:1–17. doi: 10.1038/s41398-019-0552-0

13. Orlich MJ, Singh PN, Sabaté J, Jaceldo-Siegl K, Fan J, Knutsen S, et al. Vegetarian dietary patterns and mortality in adventist health Study 2. *JAMA Intern Med.* (2013) 173:1230–8. doi: 10.1001/jamainternmed.2013.6473

14. Rosell M, Appleby P, Spencer E, Key T. Weight gain over 5 years in 21 966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. *Int J Obes.* (2006) 30:1389–96. doi: 10.1038/sj.ijo.0803305

15. Tonstad S, Butler T, Yan R, Fraser GE. Type of vegetarian diet, body weight, and prevalence of type 2 diabetes. *Diabetes Care.* (2009) 32:791–6. doi: 10.2337/dc08-1886

16. Kahleova H, Levin S, Barnard N. Cardio-metabolic benefits of plant-based diets. *Nutrients.* (2017) 9:848. doi: 10.3390/nu9080848

17. Karlsen MC, Rogers G, Miki A, Lichtenstein AH, Folta SC, Economos CD, et al. Theoretical food and nutrient composition of whole-food plant-based and vegan diets compared to current dietary recommendations. *Nutrients.* (2019) 11:625. doi: 10.3390/nu11030625

18. Jovanovic CE. The association of plant-based food and metabolic syndrome using NHANES 2015-2016 data. *J Public Health.* (2021). doi: 10.1093/pubmed/fdab403 [Epub ahead of print].

19. Satija A, Hu FB. Plant-based diets and cardiovascular health. *Trends Cardiovasc Med.* (2018) 28:437–41. doi: 10.1016/j.tcm.2018.02.004

20. Greger M. A whole food plant-based diet is effective for weight loss: the evidence. *Am J Lifestyle Med.* (2020) 14:500–10. doi: 10.1177/1559827620912400

21. Baden MY, Liu G, Satija A, Li Y, Sun Q, Fung TT, et al. Changes in plant-based diet quality and total and cause-specific mortality. *Circulation.* (2019) 140:979–91. doi: 10.1161/CIRCULATIONAHA.119.041014

22. Alissa EM, Ferns GA. Dietary fruits and vegetables and cardiovascular diseases risk. *Crit Rev Food Sci Nutr.* (2017) 57:1950–62.

23. Wu Y, Zhang D, Jiang X, Jiang W. Fruit and vegetable consumption and risk of type 2 diabetes mellitus: a dose-response meta-analysis of prospective cohort studies. *Nutr Metab Cardiovasc Dis.* (2015) 25:140–7. doi: 10.1016/j.numecd.2014.10.004

24. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. *JAMA.* (2017) 317:912–24. doi: 10.1001/jama.2017.0947

25. Barnard ND, Cohen J, Jenkins DJ, Turner-McGrievy G, Gloede L, Green A, et al. A low-fat vegan diet and a conventional diabetes diet in the treatment of type 2 diabetes: a randomized, controlled, 74-wk clinical trial. *Am J Clin Nutr.* (2009) 89:1588S–96S. doi: 10.3945/ajcn.2009.26736H

26. Dinu M, Abbate R, Gensini GF, Casini A, Sofi F. Vegetarian, vegan diets and multiple health outcomes: a systematic review with meta-analysis of observational studies. *Crit Rev Food Sci Nutr.* (2017) 57:3640–9. doi: 10.1080/10408398.2016.1138447

27. Song M, Chan AT, Sun J. Influence of the gut microbiome, diet, and environment on risk of colorectal cancer. *Gastroenterology.* (2020) 158:322–40. doi: 10.1053/j.gastro.2019.06.048

28. Clark M, Hill J, Tilman D. The diet, health, and environment trilemma. *Annu Rev Environ Resour.* (2018) 43:109–34. doi: 10.1146/annurev-environ-102017-025957

29. Pimentel D, Zuniga R, Morrison D. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol Econ.* (2005) 52:273–88. doi: 10.1016/j.ecolecon.2004.10.002

30. Mertens E, Kuijsten A, van Zanten HH, Kaptijn G, Dofková M, Mistura L, et al. Dietary choices and environmental impact in four European countries. *J Clean Prod.* (2019) 237:117827. doi: 10.1016/j.jclepro.2019.117827

31. Godfray HCJ, Aveyard P, Garnett T, Hall JW, Key TJ, Lorimer J, et al. Meat consumption, health, and the environment. *Science.* (2018) 361:eaam5324. doi: 10.1126/science.aam5324

32. Bonnet C, Bouamra-Mechemache Z, Réquillart V, Treich N. Regulating meat consumption to improve health, the environment and animal welfare. *Food Policy.* (2020) 97:101847. doi: 10.1016/j.foodpol.2020.101847

33. Petrovic Z, Djordjevic V, Milicevic D, Nastasijevic I, Parunovic N. Meat production and consumption: environmental consequences. *Procedia Food Sci.* (2015) 5:235–8. doi: 10.1016/j.profoo.2015.09.041

34. Statista Research Department. *Veganism And Vegetarianism In The United States-Statistics & Facts*. Hamburg: Statista Research Department (2022).

35. Lee-Kwan SH, Moore LV, Blanck HM, Harris DM, Galuska D. Disparities in state-specific adult fruit and vegetable consumption—United States, 2015. *MMWR Morb Mortal Wkly Rep.* (2017) 66:1241. doi: 10.15585/mmwr.mm6645a1

36. Global Change Data Lab. *Per Capita Meat Consumption in the United States*. Oxford: Global Change Data Lab (2021).

37. Graça J, Calheiros MM, Oliveira A. Attached to meat?(Un) Willingness and intentions to adopt a more plant-based diet. *Appetite.* (2015) 95:113–25. doi: 10.1016/j.appet.2015.06.024

38. Lea E, Worsley A. Influences on meat consumption in Australia. *Appetite.* (2001) 36:127–36. doi: 10.1006/appe.2000.0386

39. Verbeke W, Pérez-Cueto FJ, de Barcellos MD, Krystallis A, Grunert KG. European citizen and consumer attitudes and preferences regarding beef and pork. *Meat Sci.* (2010) 84:284–92. doi: 10.1016/j.meatsci.2009.05.001

40. Miki A, Karlsen M, Livingston K, Rogers G, Folta S, Lichtenstein A, et al. Motivations to adopt plant-based diets: data from the adhering to dietary approaches for personal taste (ADAPT) Study (P16-024-19). *Curr Dev Nutr.* (2019) 3(Suppl. 1):nzz050.P16-024-19. doi: 10.1093/cdn/nzz050.P16-024-19

41. Rosenfeld DL, Burrow AL. Vegetarian on purpose: understanding the motivations of plant-based dieters. *Appetite.* (2017) 116:456–63. doi: 10.1016/j.appet.2017.05.039

42. Janis IL, Mann L. *Decision Making: A Psychological Analysis Of Conflict, Choice, And Commitment*. New York, NY: Free press (1977).

43. Di Noia J, Prochaska JO. Dietary stages of change and decisional balance: a meta-analytic review. *Am J Health Behav.* (2010) 34:618–32. doi: 10.5993/ajhb.34.5.11

44. Prochaska JO, Velicer WF, Rossi JS, Goldstein MG, Marcus BH, Rakowski W, et al. Stages of change and decisional balance for 12 problem behaviors. *Health Psychol.* (1994) 13:39. doi: 10.1037/0278-6133.13.1.39

45. Plummer BA, Velicer WF, Redding CA, Prochaska JO, Rossi JS, Pallonen UE, et al. Stage of change, decisional balance, and temptations for smoking:

measurement and validation in a large, school-based population of adolescents. *Addict Behav.* (2001) 26:551–71. doi: 10.1016/s0306-4603(00)00144-1

46. Corepal R, Copeman J. The perceived barriers and benefits of consuming a plant-based diet. *Eur J Nutr Food Saf.* (2014) 4:252–3.

47. Pohjolainen P, Vinnari M, Jokinen P. Consumers' perceived barriers to following a plant-based diet. *Br Food J.* (2015) 117:1150–67. doi: 10.1002/jsfa.9624

48. Pribis P, Pencak RC, Grajales T. Beliefs and attitudes toward vegetarian lifestyle across generations. *Nutrients.* (2010) 2:523–31. doi: 10.3390/nu2050523

49. Doerksen SE, McAuley E. Social cognitive determinants of dietary behavior change in university employes. *Front Public Health.* (2014) 2:23. doi: 10.3389/fpubh.2014.00023

50. Anderson ES, Winett RA, Wojcik JR. Self-regulation, self-efficacy, outcome expectations, and social support: social cognitive theory and nutrition behavior. *Ann Behav Med.* (2007) 34:304–12. doi: 10.1007/BF02874555

51. Vadiveloo M, Lichtenstein AH, Anderson C, Aspry K, Foraker R, Griggs S, et al. Rapid diet assessment screening tools for cardiovascular disease risk reduction across healthcare settings: a scientific statement from the American Heart Association. *Circ Cardiovasc Qual Outcomes.* (2020) 13:e000094. doi: 10.1161/HCQ.0000000000000094

52. Litman L, Robinson J. *Conducting Online Research On Amazon Mechanical Turk And Beyond*. Thousand Oaks, CA: Sage Publications (2020). doi: 10.4135/9781071878804

53. Mortensen K, Hughes TL. Comparing Amazon's Mechanical Turk platform to conventional data collection methods in the health and medical research literature. *J Gen Intern Med.* (2018) 33:533–8. doi: 10.1007/s11606-017-4246-0

54. Paolacci G, Chandler J, Ipeirotis PG. Running experiments on amazon mechanical turk. *Judgm Decis Mak.* (2010) 5:411–9.

55. Pittman M, Sheehan K. Amazon's Mechanical Turk a digital sweatshop? Transparency and accountability in crowdsourced online research. *J Media Ethics.* (2016) 31:260–2. doi: 10.1080/23736992.2016.1228811

56. Kuan G, Sabo A, Sawang S, Kueh YC. Factorial validity, measurement and structure invariance of the Malay language decisional balance scale in exercise across gender. *PLoS One.* (2020) 15:e0230644. doi: 10.1371/journal.pone.0230644

57. Eshah NF, Mosleh SM, Al-Smadi A. The decisional balance toward health behaviors among patients with hypertension. *Clin Nurs Res.* (2021) 30:977–84. doi: 10.1177/1054773820967548

58. Beilei L, Yongxia M, Zhenxiang Z, Yan Z, Yongli W, Wenna W, et al. Research progress on decisional balance tools of health-related behaviors. *Chin Gen Pract.* (2020) 23:1599. doi: 10.1016/j.ypmed.2006.03.025

59. Wade R, Harper GW, Bauermeister JA. Psychosocial functioning and decisional balance to use condoms in a racially/ethnically diverse sample of young gay/bisexual men who have sex with men. *Arch Sex Behav.* (2018) 47:195–204. doi: 10.1007/s10508-016-0912-2

60. StataCorp. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC (2019).

61. Mehmetoglu M. *CONDISC: Stata Module To Perform Convergent And Discriminant Validity Assessment in CFA. Statistical Software Components S458003*. Chestnut Hill, MA: Boston College Department of Economics (2015).

62. Derbyshire EJ. Flexitarian diets and health: a review of the evidence-based literature. *Front Nutr.* (2017) 3:55. doi: 10.3389/fnut.2016.00055

63. Nunnally JC. *Psychometric Theory 3E*. New York, NY: Tata McGraw-hill education (1994).

64. Latvala T, Niva M, Mäkelä J, Pouta E, Heikkilä J, Kotro J, et al. Diversifying meat consumption patterns: consumers' self-reported past behaviour and intentions for change. *Meat Sci.* (2012) 92:71–7. doi: 10.1016/j.meatsci.2012.04.014

65. Latvala T, Niva M, Makela J, Pouta E, Heikkila J, Koistinen L, et al. Meat consumption patterns and intentions for change among Finnish consumers. *2011 International Congress, August 30-September 2, 2011*. Zurich: European Association of Agricultural Economists (2011).

66. Fresán U, Errendal S, Craig WJ. Influence of the socio-cultural environment and external factors in following plant-based diets. *Sustainability.* (2020) 12:9093. doi: 10.3390/su12219093

67. Jones N, Marks R, Ramirez R, Rios-Vargas M. *2020 Census Illuminates Racial and Ethnic Composition of the Country*. Suitland, MD: United States Census Bureau (2021).

68. United States Census Bureau. *Educational Attainment in the United States: 2020*. Suitland, MD: United States Census Bureau (2021).

69. Shrider EA, Melissa K, Frances C, Jessica S. *Income and Poverty in the United States Current Population Reports*. Suitland, MD: United States Census Bureau (2021).

70. Ornish D, Weidner G, Fair WR, Marlin R, Pettengill EB, Raisin CJ, et al. Intensive lifestyle changes may affect the progression of prostate cancer. *J Urol.* (2005) 174:1065–70. doi: 10.1097/01.ju.0000169487.49018.73

71. Smith ZL, Eggener SE, Murphy AB. African-American prostate cancer disparities. *Curr Urol Rep.* (2017) 18:1–10. doi: 10.1007/s11934-017-0724-5

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Nutritional quality and greenhouse gas emissions of vegetarian and non-vegetarian primary school meals: A case study in Dijon, France

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Since 2018 in France, national regulation mandates that school canteens serve a weekly vegetarian meal to reduce school canteens' environmental impact in addition to previous regulations imposing nutritional composition guidelines. However, a lunch without meat is often perceived as inadequate to cover the nutritional needs of children. The present study aims to assess the nutritional quality and greenhouse gas emissions (GHGE) of vegetarian and non-vegetarian school meals served in primary schools in Dijon, France. The catering department provided the composition of 249 meals served in 2019. Nutritional content and GHGE were retrieved from national food databases. The portion size of each meal component was the standard portion size recommended by the relevant French authority (GEMRCN). Meals were classified into vegetarian meals, i.e., without meat or fish ($n = 66$), or non-vegetarian meals ($n = 183$). The nutritional adequacy of the meals for children aged from 6 to 11 years was estimated using the mean adequacy ratio (MAR/2,000 kcal) as the mean percentage of daily recommended intake for 23 nutrients and the mean excess ratio (MER/2,000 kcal) as the mean percentage of excess compared to the maximum daily recommended value for three nutrients. This analysis of actual school meals shows that both vegetarian and non-vegetarian meals had a similar good nutritional quality with MAR/2,000 kcal of 87.5% (SD 5.8) for vegetarian and of 88.5% (SD 4.5) for non-vegetarian meals, and a MER/2,000 kcal of 19.3% (SD 15.0) for vegetarian and of 19.1% (SD 18.6) for non-vegetarian meals. GHGE were more than twofold reduced in vegetarian compared to non-vegetarian meals (0.9 (SD 0.3) vs. 2.1 (SD 1.0) kgCO_2 eq/meal). Thus, increasing the frequency of vegetarian meals, by serving egg-based, dairy-based or vegan recipes more frequently, would reduce GHGE while maintaining adequate nutritional quality of primary school meals.

KEYWORDS

school canteen, meals, children, nutritional quality, greenhouse gas emissions, sustainability, vegetarian

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Introduction

Non-communicable diseases (e.g., overweight, obesity, diabetes, and cardiovascular diseases) as well as environmental threats (e.g., global warming, atmospheric pollution, water pollution and deforestation) require identifying dietary changes that will improve nutritional quality and reduce the environmental impact of diets (1, 2). Because school canteens may contribute to establishing social norms around eating and account for a significant share of food consumed by children, they could act as a lever toward more sustainable food systems, i.e. by making nutritious and environmentally friendly meals accessible to a large number of children through national or local public policies (3). Hence, modification of school catering taking environmental issues into account while maintaining a strong emphasis on nutrition now seems necessary (4–7) and possible, as optimization studies identified nutritionally adequate and environmentally friendly school meals (8, 9). In Spain, the municipality of Barcelona introduced low-carbon meals in public schools during the 2020–2021 school year. The evaluation of this experiment showed that the transition to a low-carbon meal had environmental benefits by halving the environmental impacts (10). In Bahia (Brazil), the Sustainable School Program (SSP) implemented low-carbon meals twice a week in 155 schools in 4 municipalities and showed a 17% reduction in diet-related greenhouse gas emissions (GHGE) (11).

Currently, in France, 8.5 million children aged 3 to 17 years eat at least once a week at school canteens. Among children aged 3 to 10 years, 58% eat lunch regularly at the school canteens, i.e., at least 4 days a week (12). In France, the responsibility for serving meals in primary schools lies with the municipalities. Meals can be provided by municipalities services or delegated to a catering company. Since the first "National Health and Nutrition Plan" (13) was launched in 2001 in France, primary school canteens have been targeted by public health measures. School meals are typically structured based on four or five components: starter (optional), protein dish, side dish, dairy product, and dessert (optional). Each day, a unique menu is proposed to children (14)[1]. In 2011, the Ministry of Agriculture published 15 mandatory recommendations based on work of the "Market Research Group for Collective Catering and Nutrition" (GEMRCN) related to the frequency of serving dishes over 20 consecutive meals and the portion sizes based on children's age (15)[2]. A study published in 2016 by Vieux et al. demonstrated that when the 15 French recommendations were met, a 20-meal sequence covered, on average, 36% of energy daily needs and 50% of essential nutrient needs for primary school children (16). A simulation based on a sample of 40 series of 20 meals showed that nutritional quality increased with the number of respected recommendations. When the recommendations were not followed, a risk of deterioration in the nutritional quality of meals emerged (16). This study highlighted that serving only vegetarian meals would decrease the nutritional quality of the meals served to children (16). However, this result may be partly due to the lack of variety in vegetarian dishes considered in this study ($n = 41$ among the 800 dish options).

In 2018, a law for the "balance of trade relations in the agricultural and food sector and sustainable healthy food accessible to all" (EGalim) was adopted in France (17)[3]. This law contains measures related to school catering that aim at promoting sustainable school meals by increasing the proportion of organic products up to 20%, limiting the use of plastic, preventing food waste, strengthening transparency and diversifying protein sources of meals with one vegetarian meal per week, i.e. without fish or meat (18)[4]. In 2021, the Climate and Resilience law amended the objectives of the EGalim law by encouraging more vegetarian meals up to a daily vegetarian option at primary school canteens (19)[5]. This context raises questions about the acceptability of vegetarian meals at school canteens in the French context, where meat has a central place in meal composition (20). The general council for food, agriculture and rural areas (governmental organization) issued an evaluation report on the weekly vegetarian school meal in March 2021 highlighting reluctance toward this measure that was perceived by part of the population as an attack on French tradition and gastronomy, in which vegetables usually appear as a side dish (21). The view that "a meal without meat is not a real meal" also seems to be widely shared among school catering actors. It also raises the question of potential degradation of nutritional quality, as suggested by Vieux et al. (16), although nutrient profiling methods have shown that vegetarian and non-vegetarian main dishes offered in primary schools in France were generally of good nutritional quality (22). In addition, a recent simulation study based on a database of 2,316 school dishes demonstrated that the best trade-off for decreasing the environmental impacts of school meals without altering their nutritional quality was a frequency of 12 vegetarian meals over a total of 20 meals (23).

Reduction of meat consumption in school canteens is key to building more environmentally friendly food systems, but

Abbreviations: ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; GHGE, greenhouse gas emissions; MAR, mean adequacy ratio; MER, mean excess ratio; MRV, maximum recommended value; LA, linoleic acid; RDI, recommended daily intake; SFA, saturated fatty acids; SSP, sustainable school program.

1 https://www.education.gouv.fr/la-restauration-scolaire-6254

2 https://www.economie.gouv.fr/daj/recommandation-nutrition

3 https://www.legifrance.gouv.fr/loda/id/JORFTEXT000037547946/

4 https://agriculture.gouv.fr/egalim-tout-savoir-sur-la-loi-agriculture-et-alimentation

5 https://ma-cantine.beta.gouv.fr/blog/10/

evidence based on the analysis of school meals from real life is still needed to convince catering actors, parents and children of the nutritional quality of vegetarian meals. The challenge is thus to demonstrate that a high level of nutritional quality could be maintained while limiting the negative impacts of meals on the environment and, in particular, global warming.

In the present study, we used validated indicators to compare the nutritional quality and environmental impact of all meals (66 vegetarian and 183 non-vegetarian) that were served in 2019 to children aged from 6 to 11 years old in primary schools in Dijon, France. We hypothesized that vegetarian and non-vegetarian meals would have similarly good nutritional quality, whereas the environmental impact would be significantly reduced for vegetarian meals compared to non-vegetarian meals. Moreover, we aimed at analyzing more closely the nutritional quality and environmental impact of school meals based on the type of protein dish.

Materials and methods

Data collection and database information

This study focused on meals served in 2019 (January to December) to children in primary schools in Dijon, France ($n = 249$). In this context, school meals delivery is organized by the municipality service. The central kitchen of Dijon produces and serves 4,000 meals every day to children aged 6 to 11 years (plus 4,000 for preschool aged children and municipality staff) distributed among the 38 primary school canteens (24)[6]. Meals were developed by a dietician and respected the GEMRCN frequencies. The central kitchen provided the list of the food items included in each dish analyzed in this study. An example of a meal series for 1 month is available in the Supplementary Table S1.

Three databases were created from the collected information: the meal database, the dish database and the recipe database (data are available here: https://osf.io/fk7cq/). The meal database contained the 249 meals served in 2019 corresponding to five meals served per week (excluding weekend) during the school period and school holidays. A meal was composed of five or four components, among a starter, protein dish, side dish (e.g., fries, starches, vegetables or legumes), dairy product, and dessert, with a 30- or 40-g portion of bread. Meals were split into two main categories: vegetarian ($n = 66$, all components without meat or fish) and non-vegetarian ($n = 183$).

The dish database contained 434 distinct dishes, including 65 starters, 129 protein dishes, 60 sides, 69 dairy products and 71 desserts. Each dish had a name and a code. All dishes were classified as vegetarian (without meat or fish) or non-vegetarian. Moreover, the meals were categorized based on the subcategory of the protein dishes. Five different subcategories were defined based on their level of GHGE (25), from the most to the least emitting: beef, veal and lamb; pork and poultry; fish; eggs and/or cheese; and vegan (i.e., plant-based foods only). Each dish was composed of one of several food items (e.g., pork and curry sauce, eggs and mayonnaise).

The 433 food items were included in the recipe database and identified by a name and a code. The portion size of each food item (in grams) was established by the central kitchen and closely related to the GEMRCN recommendations for primary school children (15). All food items had an edible portion equal to one except for melon (0.7), banana (0.8), clementine (0.7), tangerine (0.7), orange (0.8) and chicken thigh (0.8).

The nutritional composition of each food item was estimated using two French food nutrient composition reference tables: CIQUAL 2020 and CALNUT 2020 (26)[7]. The GHGE (in kilograms of CO_2 equivalent) of each food item was based on the AGRIBALYSE v3.0 table providing reference data on the environmental impacts of agricultural and food products obtained through life cycle analysis, comprising the following contributions to GHGE: agriculture, transport and packaging (27)[8]. Interoperability between the CIQUAL and AGRIBALYSE databases (same food products with same coding system) makes it possible to record both nutritional and environmental footprint information. A dietician performed the pairing of each food item from the central kitchen with items from these two databases. The confidence level of pairing was defined by rules available in the Supplementary Table S2. A total of 340 food items were paired at the highest confidence levels (one or two), representing approximately 79% of all recipes. For 224 food items, at least one nutrient was missing in the CIQUAL database, and imputed data from the CALNUT database were used.

Nutritional quality and environmental footprint

Total weight, energy content, nutritional quality and environmental footprint indicators were calculated at the meal level. The environmental footprint was estimated by greenhouse gas emissions (GHGE, in $kgCO_2eq$), the best-known and most-used climate change indicator (28, 29). This indicator corresponds to the modification of climate affecting the global ecosystem. The calculation was based on the theoretical portion sizes of the food items supplied by the central kitchen for children in primary school (6 to 11 years old). GHGE was

6 http://www.dijon.fr/Dijon-au-quotidien/Enfance-petite-enfance/Cantine-Inscription

7 https://ciqual.anses.fr/

8 https://doc.agribalyse.fr/documentation-en/

calculated for each food item using Equation (1) and then summed at the dish level and at the meal level.

$$GHGE_{food\ item}\ (kgCO2eq) = portion\ (g) \times \frac{GHGE_{Agribalyse}\ (kgC02eq/kg)}{1000\ (g)} \quad (1)$$

Similarly, the contents for 26 nutrients were calculated for each food item using Equation (2) and then summed at the dish level and at the meal level.

$$Nutrient\ content_{food\ item} = portion\ (g) \times edible\ portion \times \frac{nutrient\ content\ CIQUAL\ (g/100g)}{100\ (g)} \quad (2)$$

To estimate the overall nutritional quality of the meal, we used the mean adequacy ratio (MAR) which estimates the average content of several nutrients expressed as a percentage of recommended intakes (16). In the present study, MAR was calculated by taking into account 23 nutrients (proteins, fibers, vitamins B1, B2, B3, B6, B9, B12, C, D, E, A, calcium, potassium, iron, magnesium, zinc, copper iodine, selenium, linoleic acid, alpha-linolenic acid, docosahexaenoic acid), expressed as the percentage of adequacy for 2,000 kcal of a meal, as indicated in Equation (3) (16). MAR/2,000 kcal represents the nutritional quality if a meal was scaled-up to provide the daily energy requirement of 2,000 kcal. The recommended daily intakes (RDI) for the 23 nutrients are available in the Supplementary Table S3 and were obtained by weighting the recommended dietary allowance in France for sex and age range (30) based on the age and sex representativeness in children aged 4–13 years attending primary school following the method proposed by Vieux et al. (16).

$$MAR/2000\ kcal = \frac{1}{23} \times \sum_{n=1}^{23} \frac{\frac{content_n}{nrj} \times 2000}{RDI_n} \times 100 \quad (3)$$

The MAR/2,000 kcal is reported on a scale from 0 to 100%, where 100% indicates that the daily requirements for all the nutrients were met. Each ratio was truncated to 1 so that a large quantity of one nutrient could not compensate for a small quantity of another, hence nutrient coverage beyond children's daily needs was not considered.

We also calculated the mean excess ratio (MER) which estimates the excess compared to the daily maximum recommended values (MRV) of three nutrients that should be limited: saturated fatty acids (SFA), salt and total sugars. MER was expressed as the percentage of excess compared to the MRV for 2,000 kcal of a meal, as indicated in Equation (4) (31). The MRV are available in the Supplementary Table S3 and were based on French recommendations for children aged 4–12 years (32).[9, 10]

$$MER/2000\ kcal = \left[\frac{1}{3} \times \left(\sum_{n=1}^{3} \frac{\frac{content_n}{nrj} \times 2000}{MRV_n} \times 100\right)\right] - 100 \quad (4)$$

The MER/2,000 kcal is reported in percentage of excess, where 0% indicates that none of the three nutrient limits were reached. Each ratio was limited to 1, so that a small quantity of one nutrient could not compensate for a large quantity of another.

Statistical analyses

Statistical analyses were conducted after computation of the data at the meal level, as explained above. Correlations between total weight of meals, energy content, GHGE, MAR/2,000 kcal and MER/2,000 kcal were computed with Pearson correlation coefficients. To compare vegetarian meals and non-vegetarian meals, two-sample Student's t-tests were performed on weight, energy, GHGE, MAR/2,000 kcal, MER/2,000 kcal and contents in 26 nutrients per 2,000 kcal of a meal. For each nutrient and each type of meal, we also compared the percentage of RDI or MRV for 2,000 kcal of meal to the target value (100%) using one-sample Student's t-tests. Then, we performed ANOVA models to compare outcome variables (weight, energy, GHGE, MAR/2,000 kcal, MER/2,000 kcal and the nutrient contents) between the five subcategories of meals based on their protein dish (beef, veal and lamb; pork and poultry; fish; eggs and/or cheese; and vegan) and pairwise *post hoc* comparisons were performed.

Statistical analyses were performed with SAS software version 9.4. The level of significance was set to 0.05 for all of the analyses and Bonferroni correction was used to control for multiple testing across the 26 nutrients (i.e., 0.05/26 = 0.002).

Results

Description of school meal nutritional quality and environmental impact

Data from 249 meals were considered for the present analysis. The average weight of a meal was 464 g (SD 64), and the average energy content was 659 kcal (SD 125) representing 33% of the recommended daily energy intake for children aged 6 to 11 years old (i.e., 2,000 kcal). The average greenhouse gas emissions (GHGE) of a meal was 1.8 kgCO_2 eq (SD 1.0). The

9 ANSES. *Le sel* (2012). Available online at: https://www.anses.fr/fr/content/le-sel (accessed September 1, 2022).

10 ANSES. *Les lipides* (2021). Available online at: https://www.anses.fr/fr/content/les-lipides (accessed September 1, 2022).

average MAR/2,000 kcal was 88.3% (SD 4.9), indicating that on average 88% of the RDI for the 23 nutrients would be covered by 2,000 kcal of a school meal. The average MER/2,000 kcal was 19.1% (SD 17.7) indicating that on average the MRV for three nutrients (SFA, salt and total sugars) would be exceeded by 19% with 2,000 kcal of a school meal.

Pearson's correlations showed a positive association between weight and energy content and a negative association between weight and MER/2,000 kcal highlighting that the content in nutrients to limit was lower in school meals of higher weight. There was also a negative association between energy content and MAR/2,000 kcal indicating that school meals of better nutritional quality were also those of lower energy content. No association was found between GHGE and weight, energy content nor nutritional quality of the meals (Table 1).

Comparisons between non-vegetarian and vegetarian meals

Among the 249 meals served in 2019 in Dijon school canteens, 183 were non-vegetarian (73.5%), and 66 were vegetarian (26.5%), which is greater than the recommended frequency of one per week (i.e., 20%) established by the EGalim law. The nutritional quality and environmental impact of all meals ($n = 249$), non-vegetarian ($n = 183$) and vegetarian meals ($n = 66$) are shown in Table 2. Non-vegetarian and vegetarian meals had similar weights and energy contents. GHGE was significantly twofold reduced for vegetarian meals compared to non-vegetarian meals. On average, a vegetarian meal emitted 0.9 kgCO_2eq (SD 0.3), whereas a non-vegetarian meal emitted 2.1 kgCO_2eq (SD 1.0). MAR/2,000 kcal for non-vegetarian and vegetarian meals was not significantly different, likewise for MER/2,000 kcal.

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Nineteen of 23 nutrients were found in non-limiting quantities (i.e., average content ≥100% RDI) in both vegetarian and non-vegetarian meals: proteins, fibers, vitamins B1, B2, B3, B6, B9, B12, E, A, potassium, iron, magnesium, zinc, copper, iodine, selenium, linoleic acid (LA) and docosahexaenoic acid (DHA) (Table 2). In contrast, the coverage of nutritional needs was insufficient (i.e., average content <100%) for two nutrients in both types of meals: calcium and alpha-linolenic acid (ALA). Vitamin C and D were found in deficit specifically in vegetarian meals. Regarding nutrients to limit, the content was above the MRV and not statistically different in vegetarian and non-vegetarian meals for SFA (117.3% of MRV) and salt (114.2% of MRV). Values for total sugars was below the maximum limit for non-vegetarian (81.2%) and vegetarian meals (93.5%).

Comparisons of the meals from the five protein dish subcategories

The nutritional quality and environmental impact across the five meal subcategories based on protein dish: beef, veal, lamb ($n = 56$); pork and poultry ($n = 68$); fish ($n = 55$); eggs and/or cheese ($n = 40$); and vegan ($n = 30$) are shown in Table 3. Meals had the same energy content across all subcategories [$F_{(4, 244)} = 1.01$, $p = 0.404$] despite different weights [$F_{(4, 244)} = 4.40$, $p = 0.002$]. Meals with vegan or fish-based main dishes had a significantly higher weight than meals with beef, veal or lamb dishes. A significant effect of the meal subcategory on GHGE [$F_{(4, 244)} = 261.64$, $p < 0.001$], MAR/2,000 kcal [$F_{(4, 244)} = 3.81$, $p = 0.005$] and MER/2,000 kcal [$F_{(4, 244)} = 5.92$, $p < 0.001$] was noted. GHGE differed between all five subcategories: meals with beef-, veal- or lamb-based dishes emitted the most GHGE followed by meals with fish-based dishes, meals with pork- or poultry-based dishes, meals with eggs and/or cheese-based dishes, and finally vegan dishes, with an approximately five-fold reduction compared to ruminant meat-based meals. MAR/2,000 kcal was

TABLE 1 Pearson's correlation coefficients between weight, energy content, GHGE, MAR/2,000 kcal and MER/2,000 kcal of the Dijon school meals in 2019 ($n = 249$).

		GHGE	Energy content	MAR/2,000 kcal	MER/2,000 kcal
Weight	*r*	−0.06	0.27	0.01	−0.174
	p	0.307	< 0.001	0.86	0.006
GHGE	*r*		0.01	−0.001	0.004
	p		0.808	0.993	0.955
Energy content	*r*			−0.399	−0.053
	p			<0.001	0.411
MAR/2,000 kcal	*r*				0.249
	p				<0.001

TABLE 2 Nutritional quality and environmental impact of all meals ($n = 249$), non-vegetarian ($n = 183$) and vegetarian meals ($n = 66$) served in the Dijon school canteens in 2019.

	RDI[1] or MRV[2]	Mean (SD)			p^3
		All meals ($n = 249$)	Non-vegetarian meals ($n = 183$)	Vegetarian meals ($n = 66$)	
Weight (g)		464.0 (64)	463.2 (63.8)	466.3 (65.0)	0.734
Energy (%)	2,000 kcal	33.0 (6.2)	33.0 (6.3)	33.0 (5.9)	0.950
GHGE (kgCO2eq)		1.8 (1.0)	2.1 (1.0)	0.9 (0.3)	<0.001
MAR/2,000 kcal (%)		88.3 (4.9)	88.5 (4.5)	87.5 (5.8)	0.152
MER/2,000 kcal (%)		19.1 (17.7)	19.1 (18.6)	19.3 (15.0)	0.921
Proteins (%RDI)	25 g	373.6 (107.3)	400.1 (105.2)	300.1 (74.2)	<0.001
Fibers (%RDI)	13 g	215.0 (77.7)	199.1 (67.5)	259.1 (87.0)	<0.001
Vitamin B1 (%RDI)	0.8 mg	132.5 (75.7)	137.3 (83.9)	118.9 (43.1)	0.090
Vitamin B2 (%RDI)	1.2 mg	105.8 (43.9)	106.8 (40.1)	102.9 (53.2)	0.544
Vitamin B3 (%RDI)	9 mg	180.7 (98.2)	206.2 (95.3)	109.7 (66.6)	<0.001
Vitamin B6 (%RDI)	1 mg	174.3 (69.9)	182.1 (70.9)	152.4 (62.8)	0.002
Vitamin B9 (%RDI)	201 μg	181.6 (95.4)	160.4 (64.8)	240.4 (134.9)	<0.001
Vitamin B12 (%RDI)	1.4 μg	325.2 (312.0)	387.8 (339.6)	151.5 (82.8)	<0.001
Vitamin C (%RDI)	89 mg	**80.7 (63.9)**	85.2 (68.0)	**68.2 (49.3)**	0.065
Vitamin D (%RDI)	5 μg	87.5 (75.0)	92.3 (82.6)	**74.0 (45.5)**	0.089
Vitamin E (%RDI)	9.1 mg	141.1 (58.7)	133.0 (56.2)	163.6 (59.8)	<0.001
Vitamin A[4] (%RDI)	501 μg	286.5 (313.1)	291.5 (312.0)	272.4 (318.1)	0.676
Calcium (%RDI)	924 mg	**81.2 (27.1)**	**78.4 (26.6)**	**89.1 (27.3)**	0.007
Potassium (%RDI)	2892 mg	100.3 (25.3)	103.4 (25.9)	91.6 (21.5)	<0.001
Iron (%RDI)	8.2 mg	122.0 (49.1)	119.5 (51.5)	129.1 (41.4)	0.174
Magnesium (%RDI)	203 mg	139.7 (42.1)	136.6 (42.1)	148.2 (41.1)	0.054
Zinc (%RDI)	9.2 mg	116.7 (58.5)	125.3 (64.9)	92.7 (21.9)	<0.001
Copper (%RDI)	1.2 mg	181.8 (70.4)	176.7 (78.6)	196.0 (36.4)	0.056
Iodine (%RDI)	120 μg	206.5 (96.1)	213.5 (104.3)	187.2 (65.1)	0.057
Selenium (%RDI)	39 μg	577.0 (187.4)	583.3 (193.1)	559.5 (170.8)	0.352
LA[5] (%RDI)	8.9 g	104.2 (44.3)	97.8 (44.0)	121.9 (40.3)	<0.001
ALA[6] (%RDI)	2.2 g	**59.3 (47.7)**	**62.8 (54.2)**	**49.8 (18.1)**	0.059
DHA[7] (%RDI)	152 mg	138.6 (169.9)	156.7 (194.1)	88.6 (34.8)	0.005
SFA[8] (%MRV)	26 g	**117.3 (48.6)**	**119.1 (49.9)**	**112.3 (44.6)**	0.306
Salt (%MRV)	6.5 g	**114.2 (38.8)**	**113.1 (40.4)**	**117.1 (34.0)**	0.445
Total sugars[9] (%MRV)	67.5 g	84.5 (29.7)	81.2 (29.4)	93.5 (29.0)	0.004

[1]Recommended daily intake for children aged 4–13 years attending primary school in France (29). [2]Maximum recommended value for children aged 4–12 years (31–33). [3]Mean comparison of vegetarian and non-vegetarian meals (two-sample Student's t-test, significance: $p < 0.05$ for meal indicators, $p < 0.002$ for nutrients). [4]Vitamin A = retinol + beta-carotene/6. [5]Linoleic acid. [6]Alpha-linolenic acid. [7]Docosahexaenoic acid. [8]Saturated fatty acids. [9]Total sugars = fructose + glucose + maltose + saccharose. In bold, % RDI of nutrients lower than 100%, or % MRV of nutrients higher than 100% (one-sample Student's t-test, significance: $p < 0.002$), nutrients contents are given per 2,000 kcal of a meal.

significantly lower for meals with vegan dishes compared to meals with eggs and/or cheese or fish dishes, but the difference was small (4% difference between meals with vegan dishes compared to meals with eggs and/or cheese). MER/2,000 kcal was significantly lower for meals with vegan dishes compared to fish-based meals and meals with eggs and/or cheese dishes. Only meals with vegan dishes had SFA contents below the MRV [80.5% (SD 33.0)]. Each subcategory of meals had some nutrients below the RDI or above the MRV. Nutrients in deficit (significantly below RDI) and nutrients in excess (significantly above MRV) in the five meal subcategories are presented in Figure 1.

Comparative analyses between non-vegetarian and vegetarian meals as well as the five meal subcategories based on protein dish were replicated using MAR, MER and nutritional values per meal (instead of per 2,000 kcal) to present the net intake per meal. These tables are available in Supplementary Tables S4,S5.

TABLE 3 Nutritional quality and environmental impact across the five meal subcategories based on protein dish: beef, veal, lamb ($n = 56$); pork and poultry ($n = 68$); fish ($n = 55$); eggs and/or cheese ($n = 40$); vegan ($n = 30$) served in the Dijon school canteens in 2019.

	RDI[1] or MRV[2]	Mean (SD)					p[3]
		Beef. Veal. lamb ($n = 56$)	Pork. poultry ($n = 68$)	Fish ($n = 55$)	Eggs and/or cheese ($n = 40$)	Vegan ($n = 30$)	
Weight (g)		439.5 (66.4)^{b}	472.2 (63.9)^{ab}	479.5 (54.2)^{a}	450.1 (60.4)^{ab}	481.5 (67.0)^{a}	0.002
Energy (%)		32.4 (6.3)^{a}	34.2 (6.5)^{a}	32.4 (6.1)^{a}	32.6 (6.9)^{a}	32.6 (4.4)^{a}	0.404
GHGE (kgCO2eq)		3.4 (0.8)^{a}	1.3 (0.4)^{c}	1.8 (0.4)^{b}	1.0 (0.3)^{d}	0.7 (0.2)^{e}	<0.001
MAR/2,000 kcal (%)		87.6 (3.2)^{ab}	88.4 (4.4)^{ab}	89.2 (5.3)^{a}	89.6 (6.8)^{a}	85.6 (3.8)^{b}	0.005
MER/2,000 kcal (%)		15.6 (14.8)^{bc}	19.2 (19.8)^{abc}	20.9 (19.2)^{ab}	28.4 (15.0)^{a}	9.9 (11.1)^{c}	<0.001
Proteins (%RDI)	25 g	386.9 (85.2)^{a}	408.7 (110.6)^{a}	405.8 (118.7)^{a}	335.8 (75.6)^{ab}	260.7 (47.3)^{b}	<0.001
Fibers (%RDI)	13 g	204.8 (66.3)^{b}	197.0 (73.3)^{b}	196.2 (64.2)^{b}	230.7 (91.5)^{b}	288.5 (66.2)^{a}	<0.001
Vitamin B1 (%RDI)	0.8 mg	121.6 (65.4)^{a}	164.8 (111.9)^{a}	118.8 (46.5)^{a}	120.8 (50.2)^{a}	120.1 (33.2)^{a}	0.001
Vitamin B2 (%RDI)	1.2 mg	111.5 (40.3)^{ab}	104.8 (40.3)^{ab}	98.6 (33.6)^{bc}	135.0 (55.5)^{a}	**71.5 (29.4)**^{c}	<0.001
Vitamin B3 (%RDI)	9 mg	188.6 (46.7)^{b}	270.5 (106.9)^{a}	149.8 (71.1)^{b}	132.3 (74.4)^{bc}	83.1 (34.0)^{c}	<0.001
Vitamin B6 (%RDI)	1 mg	195.5 (58.5)^{a}	197.1 (78.6)^{a}	151.2 (65.3)^{a}	152.4 (49.4)^{a}	154.0 (75.2)^{a}	<0.001
Vitamin B9 (%RDI)	201 µg	154.5 (53.4)^{bc}	152.3 (71.2)^{c}	168.8 (61.4)^{bc}	256.3 (151.3)^{a}	222.9 (98.8)^{ab}	<0.001
Vitamin B12 (%RDI)	1.4 µg	418.7 (182.9)^{b}	160.9 (56.1)^{c}	635.5 (470.3)^{a}	215.3 (72.9)^{c}	100.7 (105.7)^{c}	<0.001
Vitamin C (%RDI)	89 mg	90.1 (69.1)^{a}	**78.6 (59.0)**^{a}	82.6 (74.1)^{a}	80.8 (56.9)^{a}	**64.1 (52.4)**^{a}	0.502
Vitamin D (%RDI)	5 µg	**51.4 (35.0)**^{b}	**74.9 (45.0)**^{b}	152.4 (113.9)^{a}	94.4 (50.9)^{b}	**55.0 (36.5)**^{b}	<0.001
Vitamin E (%RDI)	9.1 mg	125.8 (52.8)^{a}	125.5 (54.0)^{a}	148.9 (58.4)^{a}	169.7 (65.3)^{a}	152.6 (54.1)^{a}	<0.001
Vitamin A[4] (%RDI)	501 µg	291.1 (316.0)^{a}	289.8 (321.8)^{a}	283.5 (291.6)^{a}	339.5 (391.6)^{a}	204.9 (185.4)^{a}	0.527
Calcium (%RDI)	924 mg	**74.9 (26.4)**^{b}	**72.6 (22.3)**^{b}	**86.1 (26.4)**^{ab}	102.7 (28.6)^{a}	**75.0 (21.6)**^{b}	<0.001
Potassium (%RDI)	2892 mg	102.6 (23.8)^{a}	103.6 (24.9)^{a}	104.7 (29.8)^{a}	**85.8 (20.5)**^{a}	99.3 (19.8)^{a}	0.002
Iron (%RDI)	8.2 mg	147.7 (66.4)^{a}	105.7 (31.2)^{b}	102.2 (37.3)^{b}	134.0 (48.5)^{ab}	131.2 (36.4)^{ab}	<0.001
Magnesium (%RDI)	203 mg	122.8 (30.4)^{b}	134.7 (38.1)^{ab}	152.2 (50.8)^{ab}	140.5 (46.3)^{ab}	158.3 (32.8)^{a}	<0.001
Zinc (%RDI)	9.2 mg	188.5 (75.1)^{a}	108.8 (35.2)^{b}	**82.3 (22.6)**^{b}	100.8 (24.7)^{b}	**84.5 (14.8)**^{b}	<0.001
Copper (%RDI)	1.2 mg	170.8 (35.7)^{a}	167.0 (35.6)^{a}	192.7 (132.0)^{a}	182.5 (34.7)^{a}	215.0 (28.5)^{a}	0.014
Iodine (%RDI)	120 µg	159.8 (56.9)^{b}	171.8 (50.2)^{b}	315.3 (119.2)^{a}	186.5 (76.0)^{b}	199.7 (51.7)^{b}	<0.001
Selenium (%RDI)	39 µg	495.1 (165.6)^{c}	543.9 (157.1)^{bc}	724.7 (187.7)^{a}	475.1 (130.6)^{c}	669.9 (150.3)^{ab}	<0.001
LA[5] (%RDI)	8.9 g	95.6 (51.7)^{b}	109.8 (37.5)^{ab}	84.9 (38.8)^{b}	112.9 (31.6)^{ab}	131.6 (49.8)^{a}	<0.001
ALA[6] (%RDI)	2.2 g	**50.9 (40.8)**^{a}	**69.1 (59.7)**^{a}	**68.6 (59.2)**^{a}	**45.7 (19.3)**^{a}	**54.1 (15.0)**^{a}	0.034
DHA[7] (%RDI)	152 mg	87.0 (177.7)^{b}	**83.8 (32.9)**^{b}	318.4 (234.3)^{a}	98.5 (46.2)^{b}	**83.3 (23.8)**^{b}	<0.001
SFA[8] (%MRV)	26 g	**121.5 (47.5)**^{a}	**113.1 (47.3)**^{ab}	**120.8 (53.9)**^{a}	**141.7 (37.5)**^{a}	80.5 (33.0)^{b}	<0.001
Salt (%MRV)	6.5 g	100.9 (32.5)^{a}	**114.7 (48.0)**^{a}	**121.7 (34.3)**^{a}	**127.1 (30.1)**^{a}	106.7 (37.5)^{a}	0.006
Total sugars[9] (%MRV)	67.5 g	83.0 (29.0)^{a}	85.7 (30.4)^{a}	74.9 (27.7)^{a}	92.1 (33.9)^{a}	91.9 (23.2)^{a}	0.032

[1]Recommended daily intake for children aged 4–13 years attending primary school in France (29). [2]Maximum recommended value for children aged 4–12 years (31–33). [3]Type III fixed effects tests of the protein dish subcategory effect in ANOVA models with weight, GHGE, MAR/2,000 kcal, MER/2,000 kcal, energy content and nutrient content as dependent variables. The same numbers indicate no significant difference between subcategories (*post hoc* pairwise comparisons, significance: $p < 0.05$ for meal indicators, $p < 0.002$ for nutrients). [4]Vitamin A = retinol + beta-carotene/6. [5]Linoleic acid. [6]Alpha-linolenic acid. [7]Docosahexaenoic acid. [8]Saturated fatty acids. [9]Total sugars = fructose + glucose + maltose + saccharose. In bold, % RDI of nutrients lower than 100%, or % MRV of nutrients higher than 100% (one-sample Student's *t*-test, significance: $p < 0.002$), nutrients contents are given per 2,000 kcal of a meal.

Discussion

The goal of the present study was to compare the nutritional quality and environmental impact of vegetarian and non-vegetarian meals served in Dijon primary school canteens in 2019. Based on the national regulation that encourages a weekly vegetarian meal in school canteens, the school catering department of Dijon exceeded the recommendation in 2019 with greater than one-quarter of all meals being vegetarian meals. The average greenhouse gas emissions of a meal (1.8 kgCO_2eq) were consistent with results found in previous studies: 1.7 kgCO_2eq (33), 1.0 kgCO_2eq (34), and 1.4 kgCO_2eq (35). The GHGE of non-vegetarian meals was on average greater than two-fold higher than the GHGE of vegetarian meals. Meals with beef, veal or lamb dishes had the highest levels of GHGE followed by meals with fish dishes, meals with pork or poultry dishes, meals

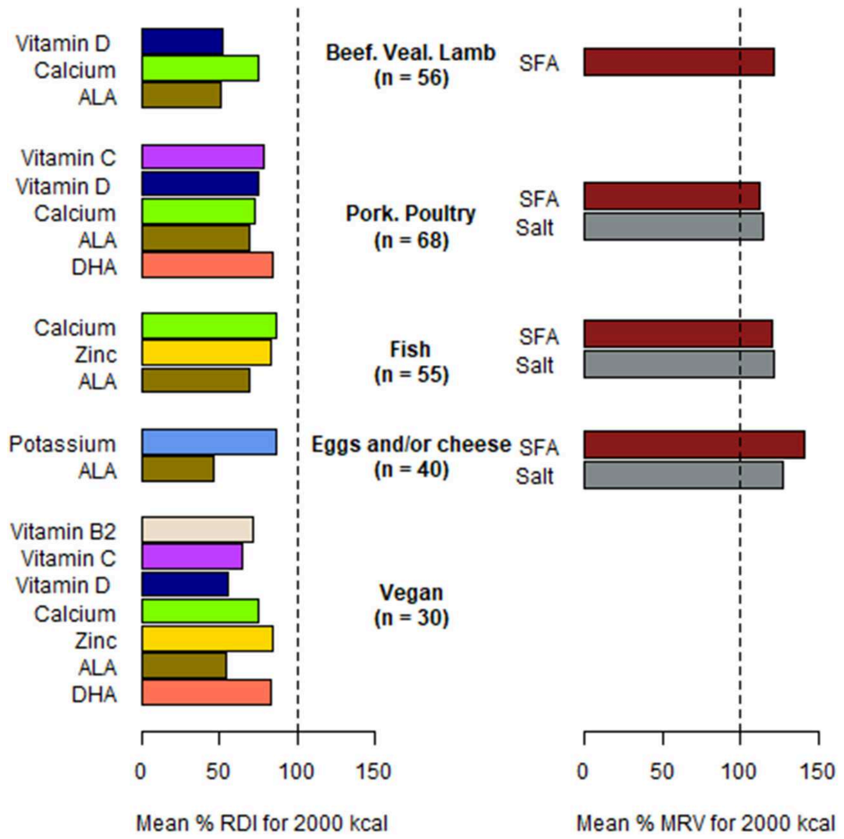

FIGURE 1
Nutrients in deficit and nutrients in excess in five meal subcategories based on protein dish served in Dijon primary school canteens in 2019. Nutrients in deficit are significantly below RDI, $p < 0.002$; Nutrients in excess are significantly above MRV, $p < 0.002$. RDI, recommended daily intake. MRV, maximum recommended value.

with eggs and/or cheese and finally vegan meals. MAR/2,000 kcal and MER/2,000 kcal of non-vegetarian and vegetarian meals were not significantly different, indicating similar adequacy with recommended daily intakes in 23 nutrients to favor and with maximum recommended values in three nutrients to limit. However, MAR/2,000 kcal and MER/2,000 kcal were significantly lower for meals with vegan dishes compared to meals with fish dishes or eggs and/or cheese dishes indicating that meals with vegan dishes had more deficits in beneficial nutrients but also less excess in harmful nutrients. For all subcategories of meals, the protein content was high, as 2,000 kcal of a meal could cover between 260.7 and 408.7% of the RDI. This finding highlights rooms for reduction of protein dish size, which would also lower GHGE. Based on the French RDI, we highlighted that nutrient deficits are generally specific to each subcategory of dishes, except for ALA content which was below the RDI for all categories of meals and calcium content which was also below the RDI for all categories of meals except eggs and/or cheese subcategory.

On average, a meal provided 659 (SD 124) kcal. This result is similar to values found in previous studies investigating the nutritional quality of school meals in France: 712 kcal (16) and 590 kcal (36). Our results confirmed the nutritional adequacy of the primary school meals served during a year in one French city; such adequacy was previously reported based on meal composition simulations (16, 22). We thus demonstrated that the willingness of French policy-makers to provide nutritionally adequate meals in school canteens (14, 18) translated into concrete results in the school.

Our data provide specific insight into how the nutritional needs of children are covered in a given school canteen organization. Based on the consumption of 7- to 10-year-old children from the French INCA3 study, some nutrients (fibers, LA, ALA, DHA, iron, vitamin D and vitamin E) have been identified as not reaching the recommendations defined by EFSA (12). Based on our results, ALA RDI were also insufficiently covered in most meals and thus dietitians may be specifically encouraged to include ALA-rich foods when developing school menus. This could be achieved without relying on supplementation, for example by including more seeds and oils with high level of omega-3. A focus on salt and SFA contents reduction would be needed as they exceeded the recommended limit in most meals. Only meals with vegan dishes did not excess the recommendation for salt and SFA and in this respect they may be served more frequently. However, this may call for a reformulation of vegan dishes to increase their beneficial nutrient densities. Overall, differences in nutrients content across the five meal subcategories were small and they provided a variety of specific nutrients, leading to high MAR/2,000 kcal values (from 85.6 to 89.6%) but for different reasons. Moreover, the five meal subcategories provided different nutrients for which coverage was higher than the RDI which could compensate for deficit in the same nutrient in other type of meals, e.g., fish-based meals were particularly rich in vitamin D [152.4% RDI (SD 113.9)] and DHA [318.4% RDI SD (234.3)]. Fish-based meals could then be encouraged to promote vitamin D and DHA intake, while ensuring fish products come from sustainable fisheries. Aquaculture generally generates less GHGE than animal farming (37) but it requires substantial energy resources and generates water pollution (38). These findings highlight the complementarity of meal subcategories in reaching overall nutritional adequacy.

Some nutrients were found in low quantities in school meals compared to the nutritional intake recommendations for children, but others excessively covered dietary needs. In particular, the protein content was high for vegetarian meals (300.1% of daily recommended intake for 2,000 kcal), even for meals with vegan-dishes based meals (260.7%); not to mention the skyrocketing rate in non-vegetarian meals (400.1%). Thus, based on our data, most school lunches cover daily protein needs in excess (>100%).

No associations were found between GHGE and the weight or energy content of the meals. This result differs from those of a study examining the relationship between the environmental impact and nutrient content of sandwiches and drinks sold in a university canteen (UK), showing that the environmental impact score was positively associated with portion sizes and calories (39). This difference may be explained by the fact that the school meal system established in France (e.g., four or five components per meal with guidelines regarding frequencies of food groups and portion sizes) strongly determines weight and energy content, thus limiting their variability but not that of GHGE.

Nor the correlation between GHGE and MAR/2,000 kcal, neither between GHGE and MER/2,000 kcal were significant. We showed that non-vegetarian meals emitted more GHGE than vegetarian meals and that the nutritional quality of non-vegetarian and vegetarian meals was similar, which may partly explain the lack of association between GHGE and indicators of nutritional quality. In a context where reducing GHGE from the food system is necessary and urgent to meet the Paris Agreement's goal of limiting the increase in global temperature to 1.5 or 2°C (40), we highlighted that lowering GHGE of school meals can be done without damaging consequences on children's health. Consistent with this idea, the results of a recent study showed that the best trade-off would be a series of 20 meals with 12 vegetarian, four fish-based and four pork- or poultry-based meals (23). Beyond school meals, recent evidence suggested that there are no specific nutritional risk in vegetarian children (41) and no clinically meaningful differences in growth or biochemical measures in vegetarian children (42).

Strengths and limitations

The present study has several limitations. First, we used nutritional and environmental indicators from CIQUAL, CALNUT and AGRIBALYSE databases that include values on average food items. We evaluated meal environmental impact based on GHGE but we did not use other indicators such as eutrophication, acidification, toxicity, biodiversity which may encompass other important impacts on our planet. Moreover, one must note that AGRIBALYSE database did not provide a distinction between organic and conventional food items, although their impacts may differ. For nutritional evaluation, we did not consider actual food items composition but average food items composition from the French reference databases. Nutrient content may vary especially for salt and fat depending on culinary practices. As the dietician from the central kitchen reported trying to limit the salt and fat content of the meals, the MER may have been overestimated for some recipes. The confidence levels for pairing with average food items were mostly high. For 92 items out of 433 with the lowest confidence levels, it would have been interesting to retrieve actual food items composition to estimate nutrient content more precisely.

The bioavailability of nutrients was not considered in the nutritional evaluation. The bioavailability of iron and zinc has been showed to be lower when they come from plant-based products (43). Bioavailability of iron (especially non-haem iron) and zinc is altered by the presence of phytates present in certain fiber-rich plants such as whole grains and pulses (44). However, bioavailability of zinc is moderately impacted even for low animal-to-plant ratio, whereas iron absorption is affected by an increase of plant products in the diet (45).

Another limitation is that we considered the food portions used by the central kitchen to estimate production needs (closely related to the GEMRCN recommendations) to calculate the nutritional quality indicators. However, the portions established by the central kitchen were probably different from the portions actually served to children and different from the portions actually consumed by children. Therefore, it would be needed to evaluate the portions of each component consumed by the children to estimate the variability in coverage of individual nutritional needs. This could be achieved by using individual food intake measure methods used in previous studies in children (46, 47). This would also provide useful insights to revise the size of the portions served as a protein dish, which may help limit food waste.

This study has several strengths. First, it was based on a unique database of meals served in one primary school canteen system during a one-year period. For the first time, the nutritional quality and GHGE were conjointly estimated for actual meals within one system. Based on the availability of yearly data, further studies could examine the evolution of nutritional quality and GHGE over several periods or seasons throughout the year or over several years. More globally, in the context of a current initiative of the Dijon catering system toward more sustainable food systems, these data provide a baseline estimation to follow the evolution of Dijon school catering during the coming years. Moreover, integrating other indicators reflecting other dimensions of sustainable food systems (48) such as attendance at school canteens, food waste, meal cost and children's liking of meals, could provide a holistic view to go toward more sustainable school meals.

Conclusion

In this study, we assessed the nutritional quality and GHGE of meals served in 2019 in Dijon primary school canteens. We showed that all meals were of good nutritional quality, notably as 2,000 kcal of a meal could cover on average 88% of the recommended daily needs for 23 nutrients. Vegetarian meals (i.e., without meat or fish) had on average two-fold lower GHGE compared with non-vegetarian meals. Thus, increasing the frequency of vegetarian meals beyond the current regulation (one per week) seems to be a good strategy to meet the double challenge of maintaining good nutritional quality and reducing the carbon impact of school catering. The school catering system of Dijon, like other municipalities, could integrate more vegetarian meals by increasing the frequency of dishes based on eggs, dairy products or vegan recipes. Future research work aimed at improving the sustainability of school catering, which would be nutritionally adequate for children and respectful of the environment, should ensure that children's habits and tastes, costs for families and for farmers are also taken into account.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://osf.io/fk7cq/.

Author contributions

Conceptualization, methodology, and writing—review and editing: JD, LM, and SN. Formal analysis and writing—original draft preparation: JD. Resources: J-MG. Data curation: JD and LM. Supervision: LM and SN. Funding acquisition: SN. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.997144/full#supplementary-material

References

1. Clark MA, Springmann M, Hill J, Tilman D. Multiple health and environmental impacts of foods. *Proc Nat Acad Sci.* (2019) 116:23357–62. doi: 10.1073/pnas.1906908116

2. Willett W, Rockström J, Loken B, Springmann M, Lang T, Vermeulen S, et al. Food in the anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet.* (2019) 393:447–92. doi: 10.1016/S0140-6736(18)31788-4

3. Coleman PC, Murphy L, Nyman M, Oyebode O. Operationalising the EAT–Lancet Commissions targets to achieve healthy and sustainable diets. *Lancet Planet Health.* (2021) 5:*e*398–e9. doi: 10.1016/S2542-5196(21)00144-3

4. Demas A, Kindermann D, Pimentel D. School meals: a nutritional and environmental perspective. *Perspect Biol Med.* (2010) 53:249–56. doi: 10.1353/pbm.0.0160

5. Fairchild R, Collins A. Serving up healthy and sustainable school meals? An analysis of school meal provision in Cardiff (UK). *J Environ Policy Plan.* (2011) 13:209–29. doi: 10.1080/1523908X.2011.578402

6. Wickramasinghe KK, Rayner M, Goldacre M, Townsend N, Scarborough P. Contribution of healthy and unhealthy primary school meals to greenhouse gas emissions in England: linking nutritional data and greenhouse gas emission data of diets. *Eur J Clin Nutr*. (2016) 70:1162–7. doi: 10.1038/ejcn.2016.101

7. Poole MK, Musicus AA, Kenney EL. Alignment Of US school lunches with The EAT-Lancet Healthy Reference diets standards for planetary health: study examines how modifying US school lunch nutrition standards could be a strategy to reduce climate change while promoting health. Health *Aff.* (2020) 39:2144–52. doi: 10.1377/hlthaff.2020.01102

8. Benvenuti L, De Santis A, Santesarti F, Tocca L. An optimal plan for food consumption with minimal environmental impact: the case of school lunch menus. *J Clean Prod*. (2016) 129:704–13. doi: 10.1016/j.jclepro.2016.03.051

9. Elinder LS, Eustachio Colombo P, Patterson E, Parlesak A, Lindroos AK. Successful implementation of climate-friendly, nutritious, and acceptable school meals in practice: the OPTIMATTM intervention study. *Sustainability.* (2020) 12:8475. doi: 10.3390/su12208475

10. Batlle-Bayer L, Bala A, Aldaco R, Vidal-Monés B, Colomé R, Fullana-i-Palmer P. An explorative assessment of environmental and nutritional benefits of introducing low-carbon meals to Barcelona schools, *Sci Total Environ.* (2021) 756:143879. doi: 10.1016/j.scitotenv.2020.143879

11. Kluczkovski A, Menezes CA, da Silva JT, Bastos L, Lait R, Cook J. An environmental and nutritional evaluation of school food menus in Bahia, Brazil that contribute to local public policy to promote sustainability. *Nutrients.* (2022) 14:1519. doi: 10.3390/nu14071519

12. ANSES. *Consommations alimentaires et apports nutritionnels dans la restauration hors foyer en France*. Maison Alfort (2021).

13. Hercberg S, Chat-Yung S, Chauliac M. The French national nutrition and health program: 2001–2006–2010, *Int J Public Health.* (2008) 53:68–77. doi: 10.1007/s00038-008-7016-2

14. Ministry of education, youth and sport. *La Restauration Scolaire.* (2020). Available online at: https://www.education.gouv.fr/la-restauration-scolaire-6254 (accessed Feb 23, 2022).

15. GEMRCN (2015). *Recommandation* nutrition. Available online at: https://www.economie.gouv.fr/daj/recommandation-nutrition (accessed Feb 23, 2022).

16. Vieux F, Dubois C, Duchêne C, Darmon N. Nutritional quality of school meals in France: impact of guidelines and the role of protein dishes. *Nutrients.* (2018) 10:205. doi: 10.3390/nu10020205

17. Légifrance. *LOI n° 2018-938 du 30 octobre 2018 pour léquilibre des relations commerciales dans le secteur agricole et alimentaire et une alimentation saine, durable et accessible à tous (1) – Légifrance.* (2018). Available online at: https://www.legifrance.gouv.fr/loda/id/JORFTEXT000037547946/ (accessed Feb 23, 2022).

18. Ministry of Food and Agriculture. *Feuille de route 2018-2022 Politique de l'alimentation.* (2018). Available online at: https://agriculture.gouv.fr/egalim-tout-savoir-sur-la-loi-agriculture-et-alimentation.

19. Ministry of Food and Agriculture. *Loi Climat et Résilience : quel impact sur les obligations EGAlim ? - ma-cantine.beta.gouv.fr.* Available online at: https://ma-cantine.beta.gouv.fr/blog/10/ (accessed Feb 23, 2022).

20. Melendrez-Ruiz J, Chambaron S, Buatois Q, Monnery-Patris S, Arvisenet G. A central place for meat, but what about pulses? Studying French consumers representations of main dish structure, using an indirect approach. *Food Res Int.* (2019) 123:790–800. doi: 10.1016/j.foodres.2019.06.004

21. CGAAER. *Évaluation de lexpérimentation du menu végétarien hebdomadaire en restauration collective scolaire*. Rapport n° 20068 (2021).

22. Poinsot R, Vieux F, Dubois C, Perignon M, Méjean C, Darmon N, et al. Nutritional quality of vegetarian and non-vegetarian dishes at school: are nutrient profiling systems sufficiently informative? *Nutrients.* (2020) 12:2256. doi: 10.3390/nu12082256

23. Poinsot R, Vieux F, Maillot M, Darmon N. Number of meal components, nutritional guidelines, vegetarian meals, avoiding ruminant meat: what is the best trade-off for improving school meal sustainability? *Eur J Nutr.* (2022). 1-6. doi: 10.1007/s00394-022-02868-1

24. Ville de Dijon. *La Cuisine* centrale (2022). Available online at: http://www.dijon.fr/Dijon-au-quotidien/Enfance-petite-enfance/Cantine-Inscription (accessed February 23, 2022).

25. Masset G, Soler LG, Vieux F, Darmon N. Identifying sustainable foods: the relationship between environmental impact. Nutritional quality, and prices of foods representative of the french diet. *J Acad Nutr Diet.* (2014) 114:862–9. doi: 10.1016/j.jand.2014.02.002

26. ANSES (2020). *CIQUAL French Food Composition* Table. Available online at: https://ciqual.anses.fr/ (accessed February 23, 2022).

27. ADEME (2020). *AGRIBALYSE®* documentation. Available online at: https://doc.agribalyse.fr/documentation-en/ (accessed February 23, 2022).

28. Röös E, Sundberg C, Tidåker P, Strid I, Hansson PA. Can carbon footprint serve as an indicator of the environmental impact of meat production? *Ecol Indic.* (2013) 24:573–81. doi: 10.1016/j.ecolind.2012.08.004

29. Ridoutt BG, Hendrie GA, Noakes M. Dietary strategies to reduce environmental impact: a critical review of the evidence base. *Adv Nutr.* 8:933–46. doi: 10.3945/an.117.016691

30. Martin A. The "apports nutritionnels conseillés (ANC)" for the French population. *Reprod Nutr Dev.* (2001) 41:119–28. doi: 10.1051/rnd:2001100

31. Vieux F, Soler LG, Touazi D, Darmon N. High nutritional quality is not associated with low greenhouse gas emissions in self-selected diets of French adults. *Am J Clin Nutr.* (2013) 97:569–83. doi: 10.3945/ajcn.112.035105

32. ANSES. Avis de l'Agence nationale de sécurité sanitaire de l'alimentation, de lenvironnement et du travail relatif á lactualisation des repères alimentaires du PNNS pour les enfants de 4 á 17, ans. Maison Alfort (2019).

33. Cerutti AK, Ardente F, Contu S, Donno D, Beccaro GL. Modelling. assessing, and ranking public procurement options for a climate-friendly catering service. *Int J Life Cycle Assess.* (2018) 23:95–115. doi: 10.1007/s11367-017-1306-y

34. De Laurentiis V, Hunt DV, Rogers CD. Contribution of school meals to climate change water use in England. *Energy Procedia.* (2017) 123:204-11. doi: 10.1016/j.egypro.2017.07.241

35. Flament, C. *Méthode de quantification des émissions de gaz à effet de serre des menus de la restauration collective Rennaise and mise en place dune évaluation des progrès réalisés, dans le cadre du Plan Alimentaire Durable Rennais*. HAL. France (2017).

36. ANSES. *Étude individuelle nationale des consommations alimentaires 3 (INCA 3), Avis de l'Anses, Rapport d'expertise collective*. Maison Alfort (2017)

37. MacLeod MJ, Hasan MR, Robb DHF, Mamun-Ur-Rashid M. Quantifying greenhouse gas emissions from global aquaculture. *Sci Rep.* (2020) 10, 11679. doi: 10.1038/s41598-020-68231-8

38. Subasinghe R, FAO. (editors). *World Aquaculture 2015: A Brief Overview*. Rome: Food and Agriculture Organization of the United Nations (FAO fisheries and aquaculture circular, no. 1140) (2017).

39. Graham F, Russell J, Holdsworth M, Menon M, Barker M. Exploring the relationship between environmental impact and nutrient content of sandwiches and beverages available in Cafés in a UK University. *Sustainability.* (2019) 11:3190. doi: 10.3390/su11113190

40. Clark MA, Domingo N, Colgan K, Thakrar S, Hill J. 'Global food system emissions could preclude achieving the 1.5° and 2°C climate change targets'. *Science* (2020) 370:705–8. doi: 10.1126/science.aba7357

41. Alexy U, Fischer M, Weder S, Längler A, Michalsen A, Sputtek A, et al. Nutrient intake and status of German children and adolescents consuming vegetarian, vegan or omnivore diets: results of the VeChi youth study. *Nutrients.* (2021) 13:1707. doi: 10.3390/nu13051707

42. Elliott LJ, Keown-Stoneman CDG, Birken CS, Jenkins DJA, Borkhoff CM, Maguire JL, et al. Vegetarian diet, growth, and nutrition in early

childhood: a longitudinal cohort study. *Pediatrics;*. (2022) 149:e2021052598. doi: 10.1542/peds.2021-052598

43. Gibson RS. The role of diet-and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. *Food Nutr Bull*. (2007) 28(1_suppl 1):S77–S100. doi: 10.1177/156482650702 81S108

44. World Health Organization and Food and Agriculture Organization of the United Nations (2004). *Vitamin and Mineral Requirements in Human Nutrition*, 2nd Edn. Geneva; Rome.

45. Perignon M, Barre T, Gazan R, Amiot MJ, Darmon N. The bioavailability of iron. zinc, protein and vitamin A is highly variable in French individual diets: impact on nutrient inadequacy assessment and relation with the animal-to-plant ratio of diets. *Food Chem.* (2018) 238:73–81. doi: 10.1016/j.foodchem.2016.12.070

46. Bouhlal S, Chabanet C, Issanchou S, Nicklaus S. Salt content impacts food preferences and intake among children. *PLoS ONE.* (2013) 8:e53971. doi: 10.1371/journal.pone.0053971

47. Diktas HE, Roe LS, Keller KL, Sanchez CE, Rolls BJ. Promoting vegetable intake in preschool children: independent and combined effects of portion size and flavor enhancement. *Appetite.* (2021) 164:105250. doi: 10.1016/j.appet.2021.105250

48. Ribal J, Fenollosa ML, García-Segovia P, Clemente G, Escobar N, Sanjuán N. Designing healthy, climate friendly and affordable school lunches. *Int J Life Cycle Assess.* (2016) 21:631–45. doi: 10.1007/s11367-015-0905-8

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Investigation of the improving effect of raw and charred hawthorn on functional dyspepsia based on interstitial cells of Cajal

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Background: Raw hawthorn (RH) is a traditional Chinese medicine commonly used to treat indigestion. Charred hawthorn (CH) is obtained from RH by charring. It is reported that the effect of CH treatment on dyspepsia is stronger than RH. However, this has not been fully proven. The purpose of this study was to compare the effects of RH and CH on functional dyspepsia (FD) model rats. And contribute to the development of dietary therapy for dyspepsia.

Methods: SPF-grade male SD rats were divided into 5 groups: the control group, the model group, the Mos group, the RH group, and the CH group. The FD rat model was established by using the methods of water restriction, fasting, tilting cage restraint, day and night upside down, swimming, and tail damping. The body weight of rats in each group was recorded. And the gastric emptying rate, intestinal propulsive rate, and the levels of motilin (MTL), gastrin (GAS), and 5-HT in serum were compared in each group. The expression of C-kit in the stomach and small intestine of each group was compared by immunofluorescence and PCR.

Results: RH and CH could increase weight, improve the gastric emptying rate and intestinal propulsive rate, and promote the secretion of motilin (MTL), gastrin (GAS), and 5-HT in the serum of FD rats. RH and CH can upregulate the expression of the characteristic protein c-kit of ICC in the stomach and small intestine of FD model rats, and the effect of CH is stronger than RH.

Conclusion: RH and CH may increase the number of interstitial cells of Cajal (ICC) in the gastrointestinal tract by upregulating c-kit expression, thus improving gastrointestinal motility in FD model rats. And compared with RH, CH has certain advantages.

KEYWORDS

charred hawthorn, raw hawthorn, interstitial cells of Cajal, functional dyspepsia, gastrointestinal motility

Introduction

Functional dyspepsia (FD) is one of the clinics' most common functional gastrointestinal diseases, but its etiology and pathogenesis are still unclear. It refers to a series of symptoms of dyspepsia in the stomach and duodenum, including epigastric pain, epigastric distention, early satiety, belching, loss of appetite, nausea, and vomiting (Tack et al., 2006). In the Roman type III diagnostic criteria, FD is divided into two subtypes, namely, postprandial discomfort syndrome and epigastric pain syndrome. Usually, in the general population, the incidence of symptoms of dyspepsia is 20%. In addition, 80% of patients were not found to have organic lesions during other tests such as endoscopy. The disease is called functional dyspepsia because there's no explanation for its symptoms (Ford et al., 2015). The overall prevalence of FD is 16% in the general population (Ford et al., 2020). FD is a difficult disease to cure clinically, and its influencing factors are many and complex. Although FD is usually not life-threatening, the quality of life, social behavior, and mental health of patients would be severely affected (Aro et al., 2011). In one survey, there was no significant difference in the effect of FD and organic dyspepsia on work efficiency (Sander et al., 2011). This suggests that FD is as harmful as organic dyspepsia.

Although FD is a common disease, the pathophysiological basis is not clear, and it may be related to gastrointestinal motility disorders, hypersensitivity reactions in the stomach, *Helicobacter pylori* infection, and psychosocial factors (Wauters et al., 2020). In response to the possible pathogenesis, many common drugs have been used to treat FD. For example, *anti-Helicobacter pylori drugs*, *proton pump inhibitors* (PPI), and prokinetic drugs (Tack and Camilleri, 2018). However, these drugs are difficult to show high effectiveness in the treatment of FD, especially since it is difficult to solve the problem of recurrent episodes of FD. Finding new targets for the treatment of FD and developing targeted drugs have become the current research hotspots (Chiarioni et al., 2018). Interstitial cells of Cajal (ICC) are a class of cells located in the muscle tissue of the gastrointestinal tract (GIT). Although they make up only 5% of the cells in the muscle tissue of the gastrointestinal tract, they play a key role in regulating smooth muscle function and gastrointestinal movement in harmony with the enteric nervous system (Huizinga et al., 2021). ICC mediates signal transmission of the gastrointestinal motor nervous system to smooth muscle. ICC is the pacemaker of gastrointestinal motility, which stimulates the rhythmic peristalsis of the gastrointestinal tract by spontaneously generated electrical slow waves. Therefore, structural and functional abnormalities of ICC are closely related to gastrointestinal motility disorders and the cause of many gastrointestinal diseases (Sanders et al., 2014; Zhang et al., 2016). FD is a representative disease of gastrointestinal motility disorder. ICC will be a possible direction to respond to FD in the future (Zhang et al., 2018).

FIGURE 1
Hawthorn.

Hawthorn refers to the ripe fruit of *Crataegus pinnatifida Bge* in the Rosaceae family (Figure 1). As a widely used Chinese herbal medicine and medicinal herb, many drugs related to hawthorn are included in the pharmacopeias of many countries (Martinelli et al., 2021). It has a long history of being used in indigestion, blood circulation and stasis, and cardiovascular diseases. The charred hawthorn (CH) was prepared from raw hawthorn (RH) by the stir-frying method which is a thermal processing method of traditional Chinese medicine. Modern studies have shown that RH has a certain role in improving digestive function. The traditional Chinese medicine records that compared with RH, CH has a stronger effect on improving digestive function. Related studies have investigated this effect, but more studies are needed to research the mechanism (Wei et al., 2019). As hawthorn, many foods have special pharmacological effects. They are useful for preventing and treating disease. Dietary therapy refers to the use of this kind of food to regulate bodily functions. In recent years, many studies have paid attention to the importance of dietary therapy because of the close relationship between food and gastrointestinal diseases (Liu et al., 2015; Pearlman and Akpotaire, 2019).

In this study, we compared the effects of RH and CH in the intervention of FD and elucidated the possible pathways for improving digestive function from the perspective of ICC. It will provide a possible solution for developing a CH-based dietary therapy for functional dyspepsia.

Materials and methods

Preparation of RH, CH, Mos

50 g of RH and CH (Tongrentang, China) were weighed and placed in the flask. They were extracted three times with 500 mL boiling water for 1.5 h each time. After filtration, the filtrate was combined and concentrated at 45°C. Add pure water and dilute to 0.3 g/ml. Mosapride citrate dispersible tablets

(Mos, Kanghong, China) were dissolved in pure water to make a solution of 0.15 mg/ml.

Animals

The Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine approved the experimental program and followed the international animal studies guidelines to minimize the pain and discomfort of the animals. Fifty SPF-grade male SD rats (180 ± 20 g) were purchased from Chengdu Dasuo Experimental Animal Co., Ltd. Forty rats were randomly selected to receive the following stimuli to establish the model: fasting and not prohibiting water for 24 h, water deprivation and not fasting for 24 h, tilting the cage for 24 h, the fixing for 2 h, day and night upside down, swimming for 5 min in 4°C water, and clamping the tail for 1 min. The rats were stimulated in random order. And the same stimulation does not occur for 2 consecutive days. After 21 consecutive molding days, they were divided into the model group, Mos group, RH group, and CH group. During the 7-day administration period, the rats in the model group were still subjected to random stimuli to maintain the model. The dosage of the Mos group as a positive drug group was 1.5 mg/kg/d. The dosage of RH and CH groups as therapeutic drug groups was 3 g/kg/d. The Control group and model group were given equal amounts of normal saline. The weight of the rats was recorded daily throughout the experiment.

All rats fasted for 12 h (free water) before the last dose. After the last dose for 40 min, they were fed a black semisolid paste (Including sodium carboxymethyl cellulose, milk powder, soluble starch, and activated carbon). About 2 ml each and the weight is recorded (W3). After 20 min, a blood sample was taken from the abdominal aorta. The serum was separated from blood by centrifugation at 4°C 3,000 r/min, and stored at −80°C. After the pylorus and cardia were ligated, the entire stomachs of the rats were separated. Then they were dried on filter paper and weighed (total weight of the stomach, W1). Cut the stomach along the great curvature of the stomach and remove the contents. Then the stomachs were weighed again (the net of the stomach, W2). After the small intestine is isolated, the length of the small intestine (L1) and the movement distance of black solid paste (L2) were measured by a ruler. Then, the stomach and small intestine tissues were fixed in the centrifuge tubes with 4% paraformaldehyde.

TABLE 1 Primer sequences designed for RT-PCR.

Gene	Primer	5′ → 3′	Size (bp)
c-kit	Forward	TGGCAAAGAAGACAACGA	363
	Reverse	CACATGGCGTCCACGGAT	
GAPDH	Forward	ACAGCAACAGGGTGGTGGAC	252
	Reverse	TTTGAGGGTGCAGCGAACTT	

Pathological changes in gastrointestinal tissue in rats

First, the stomach and small intestine tissues of rats were fixed in 4% paraformaldehyde solution for 48 h. After the tissues were dehydrated and embedded in paraffin, they were cut into 4 μm slices and sealed with neutral resin after hematoxylin and eosin (H and E) staining. Then the pathological changes in the stomach and small intestine tissues in each group were observed under a microscope.

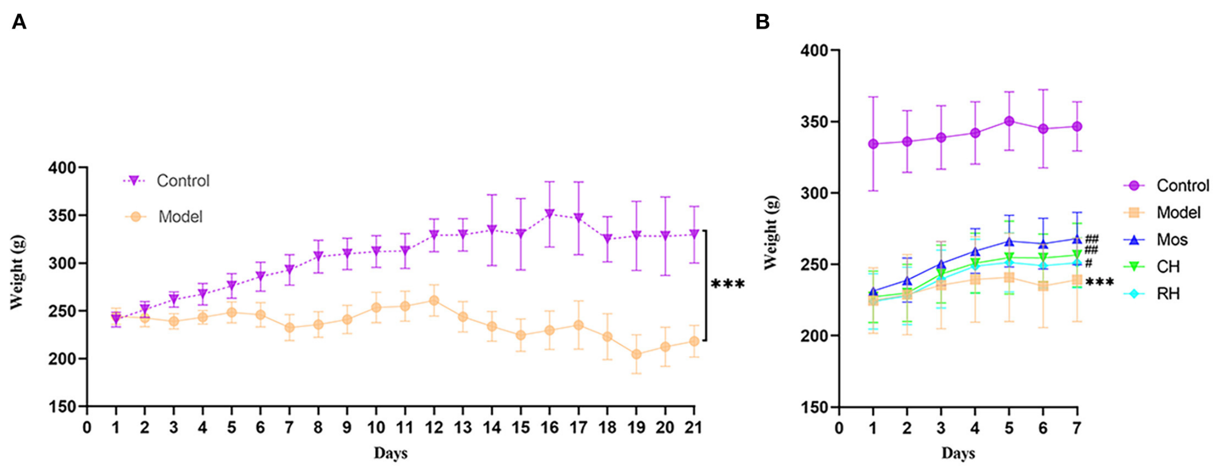

FIGURE 2
Weight changes in FD rats during modeling **(A)** and weight change during administration **(B)** (*** represents comparison with the control group: $P < 0.001$. # represents comparison with the model group: $P < 0.05$. ## represents comparison with the model group: $P < 0.01$. ### represents the comparison with the model group: $P < 0.01$).

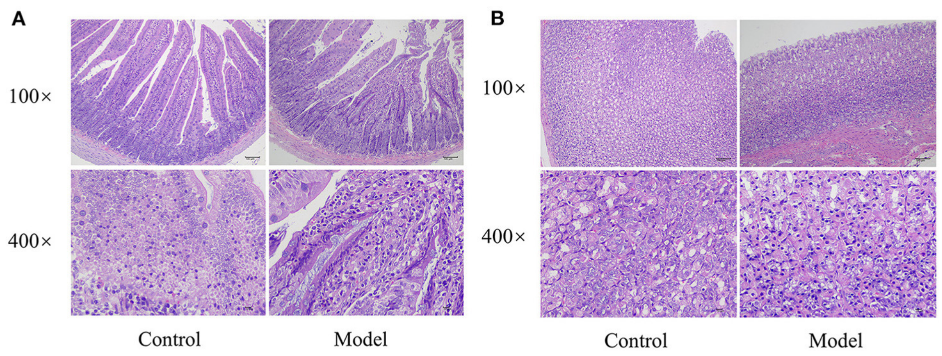

FIGURE 3
Micrographs of the small intestine **(A)** and stomach **(B)** of rats in the control group and FD model group (H and E staining).

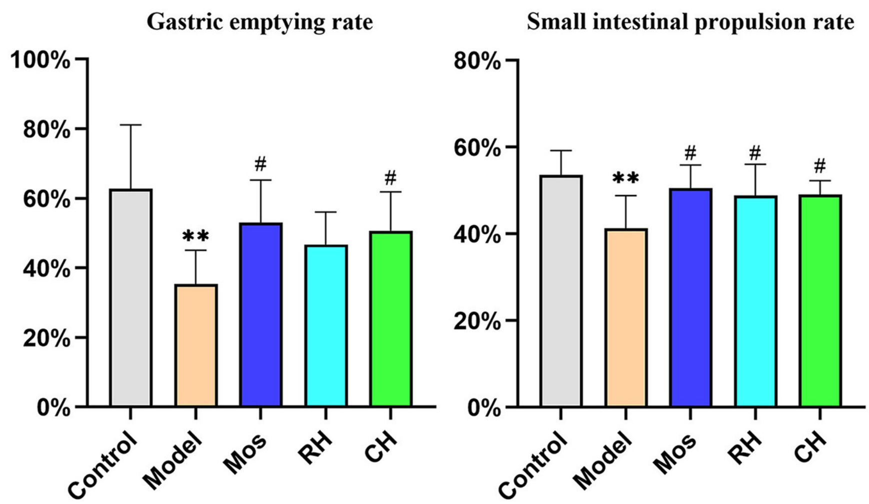

FIGURE 4
Comparison of gastric emptying rate and small intestine propulsion rate between the control, FD model, Mos group, RH, and CH group. (** represents comparison with the control group: $P < 0.01$. *represents comparison with the control group: $P < 0.05$. # represents comparison with the model group: $P < 0.05$).

Determination of intestinal propulsion and gastric emptying

The data of W1, W2, L1, and L2 can be obtained from item 2.3. The gastric emptying rate and small intestinal propulsion rate were calculated according to the formula (1-1) and (1-2) (Xiao et al., 2021).

$$\text{Gastric emptying rate (\%)} = \left(1 - \frac{W1 - W2}{W3}\right) \times 100\% \quad (1\text{-}1)$$

$$\text{Small intestine propulsion rate (\%)} = \frac{L1}{L2} \times 100\% \quad (1\text{-}2)$$

Determination of MTL, GAS, and 5-HT by ELISA

The contents of motilin (MTL, Elabscience Biotechnology, China), gastrin (GAS, Elabscience Biotechnology, China), and 5-hydroxytryptamine (5-HT, Elabscience Biotechnology, China) in the serum of rats in the control group, model group, Mos group, RH group, and CH group were determined by enzyme-linked immunosorbent assay (ELISA). According to the determination method of the commercial rat ELISA kit, the samples were incubated in 96-well-plates at 37°C for 30 min. And the washed solution was added to each well and then discarded after incubation for the 30s. The samples were operated in 96-well-plates according to the commercial rat ELISA kit. The optical density (OD) value of the sample was measured at 450 nm with a microplate reader.

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Detection of C-kit protein expression by immunofluorescence

Paraffin-embedded slides of the stomach and small intestine tissues were heated in an oven at 60°C for 1 hour. Then the slides were deparaffinized in xylene and rehydrated in ethanol series. Next, the slides were incubated in blocking buffer (1x PBS, 0.3% Triton X-100, 10% normal goat serum) for 1 h at room temperature. Then, the slides were incubated with c-kit primary antibody (1:100, A0357, ABclonal Technology, China) overnight at 4°C, washed, and incubated with Cy3-coupled goat anti-rabbit fluorescent secondary antibody for 1 h at room temperature. Images were obtained using a fluorescence microscope. Five fields were randomly selected for each sample. And the image was semi-quantized by Image J software.

Detection of expression of C-kit mRNA by real-time-quantitative polymerase chain reaction

The mRNA expression of C-kit protein in the stomach and small intestine was detected by RT-qPCR. The total RNA was isolated from the stomach and small intestine by using the Total RNA Extraction Kit (R1200, Solarbio, China). The total RNA was reverse-transcribed to cDNA with the SuperReal PreMix Plus (FP205, TIANGEN, China). The 2×SYBR Green PCR Mastermix (KR118, TIANGEN, China) was used for the amplification of cDNA. The primer sequences are shown in Table 1. The conditions of thermal cycling were 95°C for 5 min in the start cycle, 95°C for 10 s, and then a circulation was processed 40 times at 55°C for 20 s and 72°C for 30 s. A melt curve was gained by raising the temperature. In the end, cycle threshold (CT) values were obtained according to fluorescent signals. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control for normalization. The expression of c-kit mRNA was calculated with the $2^{-\Delta\Delta Ct}$ methods.

TABLE 2 Gastric emptying rate and intestinal propulsion rate in the control, FD model, Mos group, RH, and CH group.

Groups	Gastric emptying rate	Intestinal propulsion rate
Control group	62.79 ± 18.30%	53.62 ± 5.57%
Model group	35.31 ± 9.77%	41.26 ± 7.53%
Mos group	53.04 ± 12.18%	50.50 ± 5.37%
RH group	46.76 ± 9.30%	49.09 ± 3.14%
CH group	50.68 ± 11.18%	48.83 ± 7.22%

Statistical analysis

All experimental data are shown as mean ± standard deviation (SD). Statistical comparisons were made by one-way analysis of variance (ANOVA) using SPSS software (version 18.0, USA), followed by the Dunnet t multiple comparison test. The $P < 0.05$ was considered the significance level.

Results

The rats in the model group showed abnormal behavior

Before modeling, the rats had normal behaviors such as feeding and sleeping. After modeling, there was no significant change in the control group, but the rats in the model group showed a gradual loss of appetite, no increase or even decrease in body weight, and the fur color of the rats was messy and

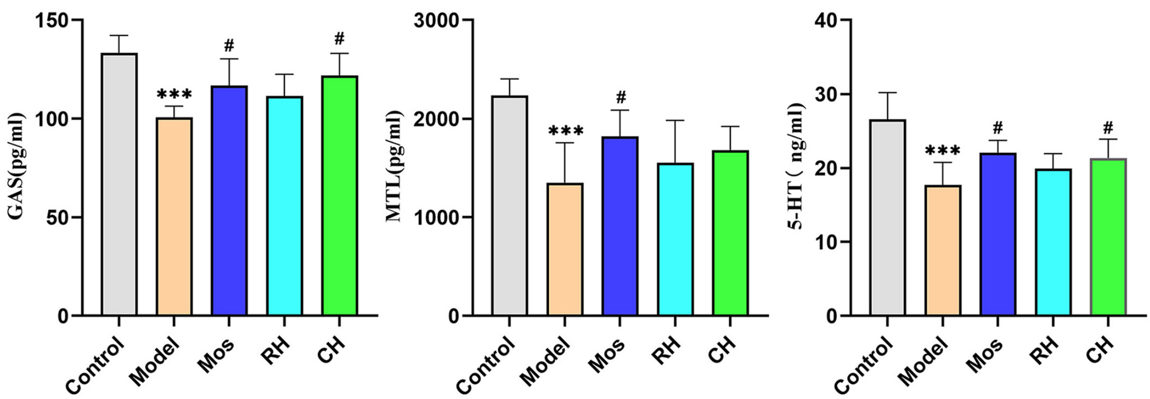

FIGURE 5
Comparison of the contents of 5-HT, GAS, and MTL in serum of rats in the control, FD model, Mos group, RH, and CH group. (*** represents comparison with the control group: $P < 0.001$. ** represents comparison with the control group: $P < 0.01$. * represents comparison with the control group: $P < 0.05$. # represents comparison with the model group: $P < 0.05$).

TABLE 3 Contents of 5-HT, GAS, and MTL in serum of rats in the control, FD model, Mos group, RH, and CH group.

Group	5-HT (ng/ml)	GAS (pg/ml)	MTL (pg/ml)
Control group	26.58 ± 3.60	133.39 ± 8.84	2236.68 ± 166.48
Model group	17.72 ± 3.40	100.69 ± 5.67	1350.92 ± 405.76
Mos group	22.08 ± 1.66	116.78 ± 13.60	1819.79 ± 265.75
RH group	19.91 ± 2.04	111.42 ± 11.08	1554.95 ± 428.00
CH group	21.37 ± 2.53	121.87 ± 11.21	1681.09 ± 240.46

TABLE 4 Expression of the c-kit protein in the stomach and small intestine of rats in the control, FD model group, Mos group, RH, and CH group.

Groups	Gastric tissue	Small intestinal tissue
Control group	34.84 ± 6.89	38.74 ± 13.61
Model group	13.79 ± 2.15	5.99 ± 3.81
Mos group	30.67 ± 4.62	14.79 ± 1.92
RH group	26.88 ± 5.99	25.46 ± 2.34
CH group	34.74 ± 8.03	31.96 ± 7.34

dim. The rats in the model group showed decreased sensitivity to external stimuli such as capture and drive.

The changes in body weight were different in each group

During modeling (Figure 2A), the weight of rats in the control group showed an increasing trend, while that in the model group showed a decreasing trend. At the end of the modeling, there was a significant difference in weight between the two groups. During the administration period (Figure 2B), the weight of rats in the control group remained unchanged, while the weight of rats in the other groups showed an increasing trend. The weight increase of rats in the Mos, RH, and CH groups was better than that in the model group, but there was still a significant difference from that in the control group.

There were no abnormal lesions in the control group and model group

According to the histological observation of the model group and the control group, the morphology of the stomach and small intestine of rats in each group were normal without obvious ulcers, bleeding, and erosion. Histological sections of each tissue sample showed that the histological structure of the stomach and small intestine was normal without obvious inflammatory cell infiltration and other pathological changes (Figure 3). The results showed that there was no organic lesion of the gastrointestinal tract in rats, indicating that the model met the requirements.

The group of three treatments could improve gastrointestinal motility in FD rats

As shown in Figure 4 and Table 2, there were significant differences between the control group and the model group ($P < 0.01$). It indicates successful modeling. Compared with the model group, the intestinal propulsion rate and gastric emptying rate of rats in the CH and Mos groups were significantly increased ($P < 0.05$). RH significantly promoted intestinal propulsion in FD rats ($P < 0.05$). However, there was no significant difference between the RH group and the model group in promoting gastric emptying.

The group of three treatments could promote the secretion of gastrointestinal hormones in FD rats

As shown in Figure 5 and Table 3, the contents of MTL, GAS, and 5-HT in the serum of the model group were significantly lower than the control group ($P < 0.001$). However, Mos, RH, and CH can interfere with their contents in the serum of FD rats. Compared with the model group, the contents of GAS increased significantly in the Mos group and CH group ($P < 0.05$), but there was no obvious upward trend in the RH group. For MTL, only the Mos group had a significant increase compared with the model group ($P < 0.05$), and there was an insignificant increase in the RH group and CH group. Compared with the model group, 5-HT increased significantly in the CH group and the Mos group ($P < 0.01$), while the RH group had an insignificant increasing trend.

Effect of Mos, RH, and CH on c-kit protein expression

The results are shown in Table 4, Figures 6, 7. Compared with the control group, the mean fluorescence density of c-kit protein in the stomach and small intestine of the model group was significantly decreased ($P < 0.001$). RH and CH can significantly increase the mean fluorescence density of c-kit protein in the small intestine of FD model rats ($P < 0.001$), but Mos has no obvious effect. The mean fluorescence density in the CH group was higher than that in the RH group. In gastric tissues, compared with the model group, the mean fluorescence density of the Mos group and CH group was significantly different ($P < 0.001$). The mean fluorescence density in gastric

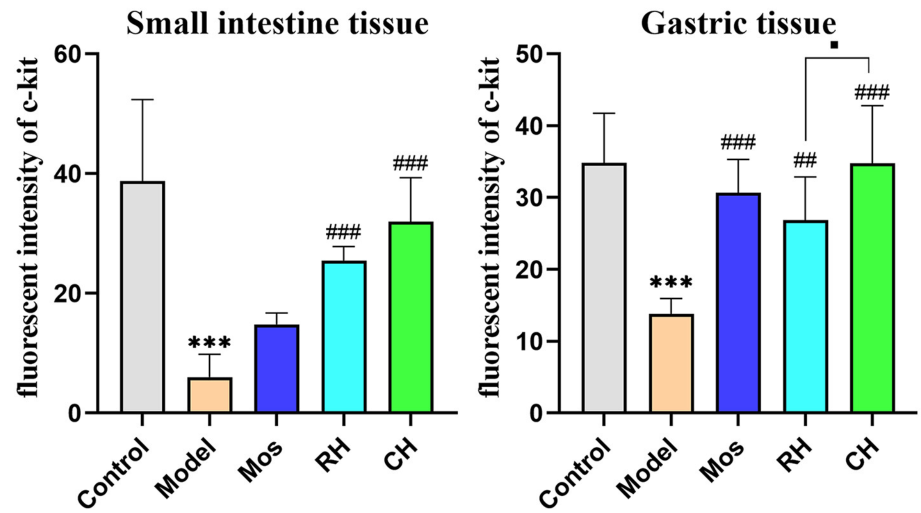

FIGURE 6
Comparison of c-kit protein expression in the stomach and small intestine of rats in the control, FD model group, Mos group, RH, and CH group. (*** represents comparison with the control group: $P < 0.001$. ## represents comparison with the model group: $P < 0.01$. ### represents the comparison with the model group: $P < 0.01$. ■ represents the comparison with the RH group: $P < 0.05$).

tissues of the RH group was also significantly different from that in the model group ($P < 0.01$), but significantly lower than that in the CH group ($P < 0.05$).

CH significantly upregulated c-kit mRNA expression in FD rats

As shown in Figure 8 and Table 5, compared with the control group, the expression of c-kit mRNA in the stomach and small intestine of the FD model group was significantly decreased ($P < 0.001$). RH, CH, and Mos could interfere with c-kit mRNA expression in the stomach and small intestine of FD rats. In small intestine tissue, CH had the most significant effect in upregulating c-kit mRNA expression ($P < 0.001$), followed by hawthorn ($P < 0.01$), and Mos was weaker than the former two ($P < 0.05$). In the gastric tissue, the effect of CH is still the most significant ($P < 0.001$), followed by Mos ($P < 0.01$), and RH had the weakest effect ($P < 0.05$).

Discussion

The traditional theory of Chinese medicine holds that "fried to brown" helps to improve digestive function and promote gastrointestinal motility. Many herbal medicines that are fried to brown are thought to promote digestion (Liu et al., 2021). Hawthorn has a long history of being used to treat indigestion, and it often appears in prescriptions related to digestive function. Some studies have shown that CH is more effective in improving digestive function than RH (Wang et al., 2019). Our study investigated the effects of RH and CH in the intervention of FD and elucidated the possible mechanism.

Establishment of FD models

There are many methods for establishing the FD model. The method of establishing the FD model in this study is improved based on the existing research (Liang et al., 2018; Zhu et al., 2020). In this study, multiple factors were used for chronic induction modeling, which is determined by the complex pathogenesis of FD. During the modeling process, the weight of rats in the control group increased gradually, while the weight of rats in the model group increased slowly or decreased occasionally. This is consistent with the clinical features of FD patients (Tack et al., 2016). No obvious pathological changes were found in the stomach and small intestine sections of the control group and the model group after H&E staining. This indicated that no organic lesions of the digestive system were caused by modeling. This is in line with the characteristics of FD and indicates that the FD rat model in this study is reasonable.

Comparison of gastrointestinal motility of rats in each group

Gastric emptying is the process by which food is pushed from the stomach into the duodenum. The gastric emptying rate can reflect the quality of gastric motility (Al-Saffar et al., 2019). The delay of gastric emptying is closely related to gastrointestinal motility (Kusano et al., 2014). The small intestine propulsion

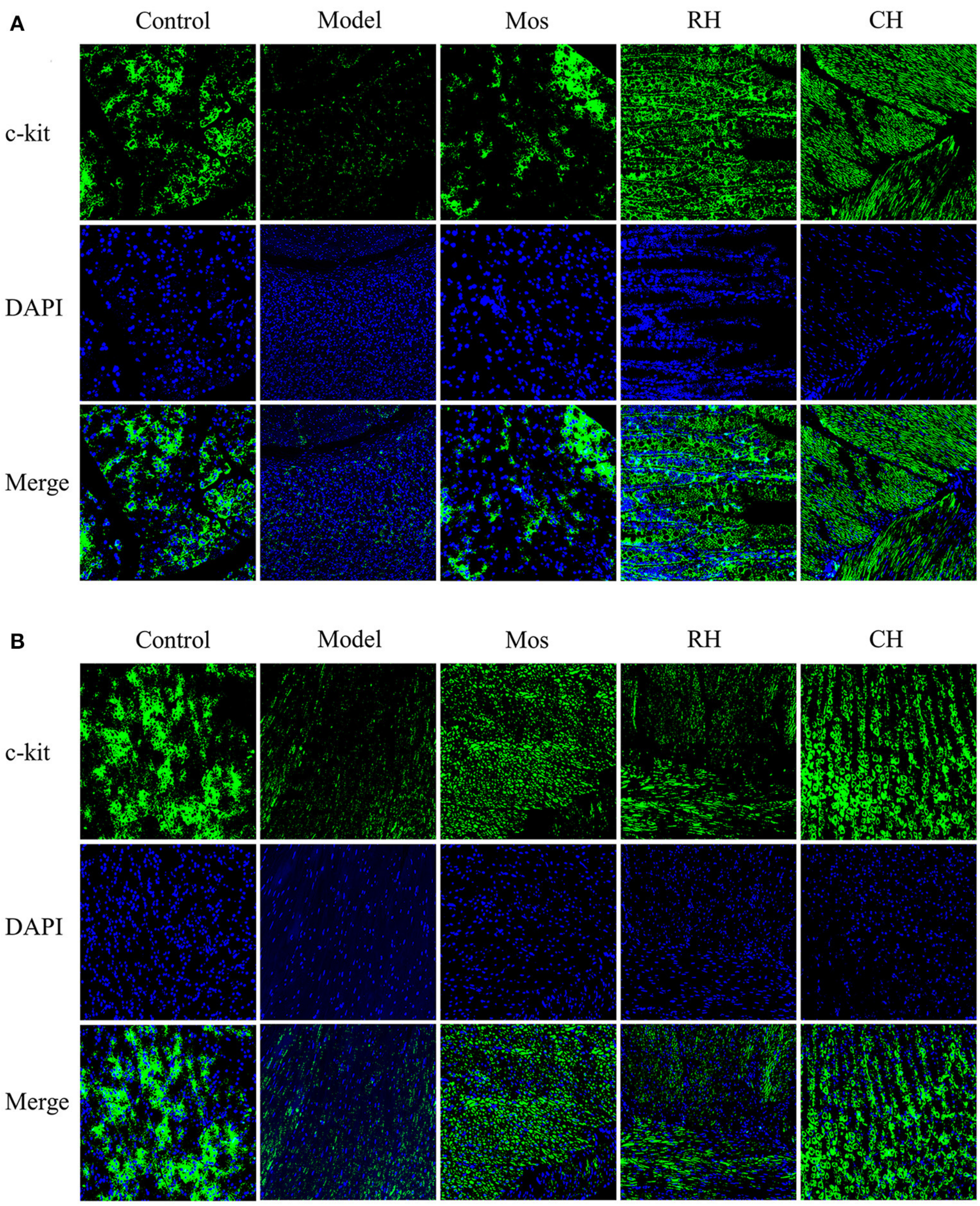

FIGURE 7
Immunofluorescence images of Characteristic protein c-kit of Cajal interstitial cells in the stomach **(B)** and small intestine **(A)** of rats in the control, FD model, Mos group, RH, and CH group.

rate can reflect the peristaltic performance of the intestine. Reduced intestinal peristalsis is consistent with the clinical symptoms of FD. In this study, gastric emptying and intestinal propulsion in FD model rats were significantly weakened after modeling. After 7 days of continuous administration, gastric emptying rate and intestinal propulsion rate were improved in Mos, RH, and CH groups. CH showed significant effects on improving intestinal propulsion and gastric emptying in FD rats. RH can also improve intestinal propulsion in FD rats, but the effect of promoting gastric emptying is weak.

Comparison of gastrointestinal hormones in serum of rats in each group

Gastrointestinal hormones secreted by gastrointestinal mucosal cells are closely related to gastrointestinal motility disorders (Mori et al., 2022). MTL is produced by endocrine cells in the intestinal mucosa. It is an important regulator of gastrointestinal motility through a specific motilin receptor (MLN-R) (Kitazawa and Kaiya, 2021). GAS is a peptide hormone mainly existing in G cells of the gastric antrum. Its main physiological function is to stimulate the secretion of gastric

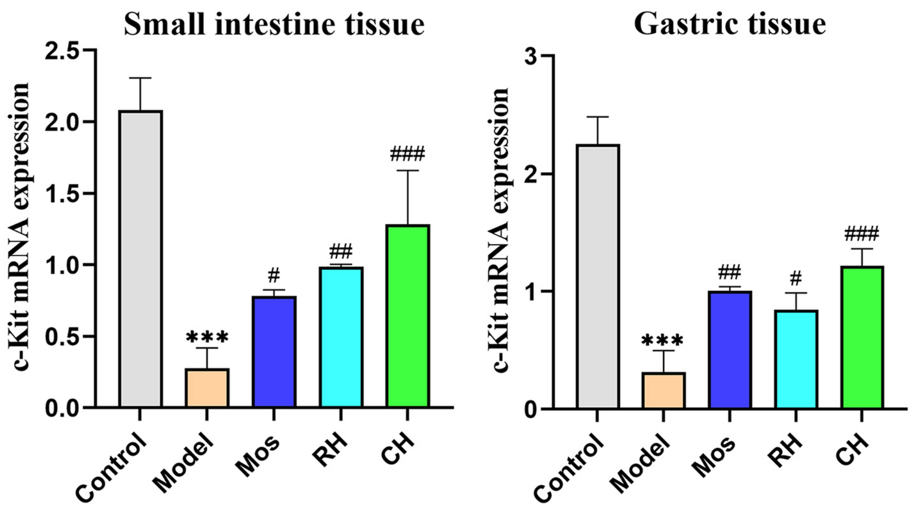

FIGURE 8
The expression of c-kit mRNA in the control, FD model, Mos group, RH, and CH group. (*** represents comparison with the control group: $P < 0.001$. # represents the comparison with the model group: $P < 0.05$. ## represents comparison with the model group: $P < 0.01$. ### represents the comparison with the model group: $P < 0.01$).

TABLE 5 The expression of c-kit mRNA in the control, FD model, Mos group, RH, and CH group.

Groups	Gastric tissue	Small intestinal tissue
Control group	2.25 ± 0.23	2.08 ± 0.23
Model group	0.31 ± 0.18	0.28 ± 0.14
Mos group	1.01 ± 0.67	0.74 ± 0.07
RH group	0.84 ± 0.14	1.01 ± 0.13
CH group	1.01 ± 0.03	1.28 ± 0.38

acid (Schubert and Rehfeld, 2019). 5-HT is synthesized, stored, and released by a subset of intestinal endocrine cells called enterochromaffin (EC) cells in the intestinal mucosa, which has been recognized as an important signaling molecule in the intestine (Mawe and Hoffman, 2013). In this study, the three gastrointestinal hormones in the FD model group showed a downregulation trend. As a positive drug, Mos can upregulate three gastrointestinal hormones in FD rats. RH and CH also have the effect to upregulate partial gastrointestinal hormones. However, in general, the effect of RH and CH on gastrointestinal hormones in FD rats was not strong, and there was no significant difference between them.

The number of ICC in the stomach and small intestine of rats in each group was compared

C-Kit protein is a quantitative marker of ICC. C-kit is a proto-oncogene that encodes receptor tyrosine kinase (kit) (Huizinga et al., 1995). The emergence of c-kit protein antibodies provides a variety of effective methods to identify ICC in pathological tissue sections (Streutker et al., 2007). The number of ICC was consistent with the expression trend of c-kit protein (Li et al., 2019). That is, the expression of c-Kit protein reflects the number of ICC changes. The results of immunofluorescence and PCR showed that the number of ICC in the small intestine and stomach of the FD model group was significantly decreased, while the three therapeutic drugs showed different effects on the number of ICC in gastrointestinal tissue. In general, the effect of hawthorn on the upregulation of ICC in the gastrointestinal tract was better than RH and Mos. RH and CH may improve gastrointestinal motility in FD model rats by regulating the number of ICC cells in the gastrointestinal tract. However, the limitation of this study is that more research is needed to prove this pathway and explain the mechanism.

Conclusion

We have proved that hawthorn decoction had an effect on improving the digestive function of FD rats, and the effect of CH was stronger than RH. CH could increase weight, improve intestinal propulsion and gastric emptying, and promote the secretion of motilin (MTL), gastrin (GAS), and 5-HT in the serum of FD rats. And the possible pathway is to improve gastrointestinal motility by increasing the number of ICC in the stomach and small intestine of FD rats. Our study will help to develop a functional food based on hawthorn and provide a possible dietary therapy for dyspepsia.

Data availability statement

The data used to support the findings of this study are available from the first author upon request.

Ethics statement

The experimental program was approved by the Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine.

Author contributions

LA and LZ conceived the project and wrote the article. CW provided supervision. LZ, QL, YT, and TC performed the research. LA, LZ, QL, YT, and CW analyzed the data. All authors have read and approved the manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Al-Saffar, A., Takemi, S., Saaed, H. K., Sakata, I., and Sakai, T. (2019). Utility of animal gastrointestinal motility and transit models in functional gastrointestinal disorders. *Best Pract. Res. Clin. Gastroenterol.* 40–41, 101633. doi: 10.1016/j.bpg.2019.101633

Aro, P., Talley, N. J., Agréus, L., Johansson, S. E., Bolling-Sternevald, E., Storskrubb, T., et al. (2011). Functional dyspepsia impairs quality of life in the adult population. *Aliment. Pharmacol. Ther.* 33, 1215–1224. doi: 10.1111/j.1365-2036.2011.04640.x

Chiarioni, G., Pesce, M., Fantin, A., and Sarnelli, G. (2018). Complementary and alternative treatment in functional dyspepsia. *United European Gastroenterol. J.* 6, 5–12. doi: 10.1177/2050640617724061

Ford, A. C., Mahadeva, S., Carbone, M. F., Lacy, B. E., and Talley, N. J. (2020). Functional dyspepsia. *Lancet* 396, 1689–1702. doi: 10.1016/S0140-6736(20)30469-4

Ford, A. C., Marwaha, A., Sood, R., and Moayyedi, P. (2015). The global prevalence of, and risk factors for, uninvestigated dyspepsia: a meta-analysis. *Gut* 64, 1049–1057. doi: 10.1136/gutjnl-2014-307843

Huizinga, J. D., Hussain, A., and Chen, J. H. (2021). Interstitial cells of Cajal and human colon motility in health and disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 321, G552–G575. doi: 10.1152/ajpgi.00264.2021

Huizinga, J. D., Thuneberg, L., Klüppel, M., Malysz, J., Mikkelsen, H. B., and Bernstein, A. (1995). W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 373, 347–349. doi: 10.1038/373347a0

Kitazawa, T., and Kaiya, H. (2021). Motilin comparative study: structure, distribution, receptors, and gastrointestinal motility. *Front. Endocrinol.* 12, 700884. doi: 10.3389/fendo.2021.700884

Kusano, M., Hosaka, H., Kawada, A., Kuribayashi, S., Shimoyama, Y., Zai, H., et al. (2014). Gastrointestinal motility and functional gastrointestinal diseases. *Curr. Pharm. Des.* 20, 2775–2782. doi: 10.2174/13816128113199990572

Li, L., Zou, C., Zhou, Z., Wang, X., and Yu, X. (2019). Phenotypic changes of interstitial cells of Cajal after intestinal obstruction in rat model. *Braz. J. Med. Biol. Res.* 52, e8343. doi: 10.1590/1414-431x20198343

Liang, Q., Yan, Y., Mao, L., Du, X., Liang, J., Liu, J., et al. (2018). Evaluation of a modified rat model for functional dyspepsia. *Saudi J. Gastroenterol.* 24, 228–235. doi: 10.4103/sjg.SJG_505_17

Liu, X., Cao, S., and Zhang, X. (2015). Modulation of gut microbiota-brain axis by probiotics, prebiotics, and diet. *J. Agric. Food Chem.* 63, 7885–7895. doi: 10.1021/acs.jafc.5b02404

Liu, Y., Liao, W., Liu, X., Hu, Y., Zhu, X., Ju, L., et al. (2021). Digestive promoting effect and mechanism of Jiao Sanxian in rats. *J. Ethnopharmacol.* 278, 114334. doi: 10.1016/j.jep.2021.114334

Martinelli, F., Perrone, A., Yousefi, S., Papini, A., Castiglione, S., Guarino, F., et al. (2021). Botanical, phytochemical, anti-microbial and pharmaceutical characteristics of hawthorn (*Crataegusmonogyna Jacq.*), Rosaceae. *Molecules* 26, 7266. doi: 10.3390/molecules26237266

Mawe, G. M., and Hoffman, J. M. (2013). Serotonin signalling in the gut–functions, dysfunctions and therapeutic targets. *Nat. Rev. Gastroenterol. Hepatol.* 10, 473–486. doi: 10.1038/nrgastro.2013.105

Mori, H., Verbeure, W., Schol, J., Carbone, F., and Tack, J. (2022). Gastrointestinal hormones and regulation of gastric emptying. *Curr. Opin. Endocrinol. Diabetes Obes.* 29, 191–199. doi: 10.1097/MED.0000000000000707

Pearlman, M., and Akpotaire, O. (2019). Diet and the role of food in common gastrointestinal diseases. *Med. Clin. North Am.* 103, 101–110. doi: 10.1016/j.mcna.2018.08.008

Sander, G. B., Mazzoleni, L. E., Francesconi, C. F., Balbinotto, G., Mazzoleni, F., Wortmann, A. C., et al. (2011). Influence of organic and functional dyspepsia on work productivity: the HEROES-DIP study. *Value Health* 14(5 Suppl 1), S126–S129. doi: 10.1016/j.jval.2011.05.021

Sanders, K. M., Ward, S. M., and Koh, S. D. (2014). Interstitial cells: regulators of smooth muscle function. *Physiol. Rev.* 94, 859–907. doi: 10.1152/physrev.00037.2013

Schubert, M. L., and Rehfeld, J. F. (2019). Gastric peptides-gastrin and somatostatin. *Compr. Physiol.* 10, 197–228. doi: 10.1002/cphy.c180035

Streutker, C. J., Huizinga, J. D., Driman, D. K., and Riddell, R. H. (2007). Interstitial cells of Cajal in health and disease. Part I: normal ICC structure and function with associated motility disorders. *Histopathology* 50, 176–189. doi: 10.1111/j.1365-2559.2006.02493.x

Tack, J., and Camilleri, M. (2018). New developments in the treatment of gastroparesis and functional dyspepsia. *Curr. Opin. Pharmacol.* 43, 111–117. doi: 10.1016/j.coph.2018.08.015

Tack, J., Ly, H. G., Carbone, F., Vanheel, H., Vanuytsel, T., Holvoet, L., et al. (2016). Efficacy of mirtazapine in patients with functional dyspepsia and weight loss. *Clin. Gastroenterol. Hepatol.* 14, 385–392.e4. doi: 10.1016/j.cgh.2015.09.043

Tack, J., Talley, N. J., Camilleri, M., Holtmann, G., Hu, P., Malagelada, J. R., et al. (2006). Functional gastroduodenal disorders. *Gastroenterology* 130, 1466–1479. doi: 10.1053/j.gastro.2005.11.059

Wang, Y., Lv, M., Wang, T., Sun, J., Wang, Y., Xia, M., et al. (2019). Research on mechanism of charred hawthorn on digestive through modulating "brain-gut" axis and gut flora. *J. Ethnopharmacol.* 245, 112166. doi: 10.1016/j.jep.2019.112166

Wauters, L., Talley, N. J., Walker, M. M., Tack, J., and Vanuytsel, T. (2020). Novel concepts in the pathophysiology and treatment of functional dyspepsia. *Gut* 69, 591–600. doi: 10.1136/gutjnl-2019-318536

Wei, Z., Ai, L., Chen, X., Li, L., Wang, L., and Fan, W., et al. (2019). Comparative studies on the regulatory effects of raw and charred hawthorn on functional dyspepsia and intestinal flora. *Trop. J. Pharm. Res.* 18, 333–339. doi: 10.4314/tjpr.v18i2.16

Xiao, H. L., Xiao, Y. J., Wang, Q., Chen, M. L., and Jiang, A. L. (2021). Moxibustion regulates gastrointestinal motility via HCN1 in functional dyspepsia rats. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 27, e932885. doi: 10.12659/MSM.932885

Zhang, G., Xie, S., Hu, W., Liu, Y., Liu, M., Liu, M., et al. (2016). Effects of electroacupuncture on interstitial cells of Cajal (ICC) ultrastructure and connexin 43 protein expression in the gastrointestinal tract of functional dyspepsia (FD) rats. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 22, 2021–2027. doi: 10.12659/MSM.899023

Zhang, L. M., Zeng, L. J., Deng, J., Zhang, Y. Q., Wang, Y. J., Xie, T. Y., et al. (2018). Investigation of autophagy and differentiation of myenteric interstitial cells of Cajal in the pathogenesis of gastric motility disorders in rats with functional dyspepsia. *Biotechnol. Appl. Biochem.* 65, 533–539. doi: 10.1002/bab.1635

Zhu, J., Tong, H., Ye, X., Zhang, J., Huang, Y., Yang, M., et al. (2020). The effects of low-dose and high-dose decoctions of fructus aurantii in a rat model of functional dyspepsia. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 26, e919815. doi: 10.12659/MSM.919815

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Oxidative stress and metabolic diseases: Relevance and therapeutic strategies

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Metabolic syndrome (MS) is a prominent cause of death worldwide, posing a threat to the global economy and public health. A mechanism that causes the oxidation of low-density lipoproteins (LDL) is associated with metabolic abnormalities. Various processes are involved in oxidative stress (OS) of lipoprotein. Although the concept of the syndrome has been fiercely debated, this confluence of risk factors is associated with a higher chance of acquiring type 2 diabetes mellitus (T2DM) and atherosclerosis. Insulin resistance has been found to play a significant role in the progression of these metabolism-associated conditions. It causes lipid profile abnormalities, including greater sensitivity to lipid peroxidation, contributing to the increased prevalence of T2DM and atherosclerosis. This review aims to cover the most recent scientific developments in dietary OS, the consequence of metabolic disorders, and their most significant clinical manifestations (T2DM and atherosclerosis). It will also emphasize the effects of dietary approaches in alleviating OS in MS.

KEYWORDS

oxidative stress, metabolic diseases, a burden on health, therapeutic approaches, LDL

Introduction

Metabolism syndrome (MS) is an international public health concern. Obesity, diabetes, dyslipidemia, elevated blood pressure, and hyperglycemia (1, 2). MS is highly complicated and has unclear pathophysiology (3). Numerous research back up the idea that oxidant/antioxidant imbalance may be crucial for its symptoms. Blood samples from MS patients had higher levels of indicators for OS and lower levels of antioxidant defenses, which may indicate an overproduction of oxidizing species *in-vivo* (4). Minimally modified low-density lipoprotein (MM-LDL) and "(completely or extensively) oxidized" LDL are the two primary classifications used to characterize oxidized LDL (ox-LDL) (5). The main distinction between the two categories is that while MM-LDL differs chemically from unmodified LDL, the LDL receptor still recognizes it, unlike most known scavenger receptors. However, none of the ox-LDL preparations are identified by the LDL receptor, only a variety of scavenger receptors (5). The content and the biological consequences of the many preparations that make up each of the two categories of ox-LDL vary greatly (5).

A drop in blood vitamin C and -tocopherol concentrations, a decline in superoxide dismutase (SOD) activity, and an increase in protein and lipid oxidation have all been linked to poor antioxidant defense in MS patients (4). On stopping OS in MS, several research is being conducted. According to recent studies, diets high in whole grain cereals, fruits, and vegetables and low in animal fat can improve one's overall health (6).

During the previous two decades, T2DM and atherosclerosis have become the world's leading causes of death in the last 20 years (7, 8). The prevalence of these diseases varies from region to region (9). Diabetes is linked to many other conditions and consequences leading to tissue and organ damage. The prevalence of heart diseases, including peripheral vascular disorders, high blood pressure, ischemic heart diseases, and atherosclerosis, is especially high (80%) in North American diabetic patients (9). It is also one of the leading causes of neuropathy, retinopathy, and nephropathy (10, 11).

According to the international diabetes federation (IDF), about 415 million people with diabetes live worldwide. The prevalence rate is 8.8 and 75% of people live in developing countries. By 2040, approximately 642 million people will be affected by T2DM (12). According to this survey, prediabetes and T2DM prevalence rates are 10.91% and 26.3%, respectively. Overall, 27.4 million people older than 20 years have diabetes.

On the other hand, atherosclerotic cardiovascular diseases (ASCVD) are a leading cause of global morbidity and mortality (13, 14). According to the World Health Organization (WHO), 17.9 million individuals died from cardiovascular diseases (CVDs) in 2019, 32% of all global deaths. Of this 32%, 85% were due to heart attack and stroke. The ratio of deaths due to CVDs was three quarters more in low and middle-income countries. Under the age of seventy, 17 million premature deaths occurred in 2019 due to non-communicable diseases. Of the 17 million deceases, 38% were caused by CVDs.

This paper aims to give a broad overview of OS's contribution to the pathophysiology of MS and other associated risk factors. In particular, it is focused on (i) the relationship between ox-LDL and metabolic disorders, (ii) dietary management for a reduction in oxidation and glycation of LDL, and (iii) dietary approaches to inhibit LDL oxidation and glycation. In addition, the global health burden of MS has also been discussed.

Burden on health system

According to research in 2015, an estimated direct or indirect cost for CVDs was $555. An estimation is that annual costs will be increased to above $1 trillion by 2035 (15). In 2015, the Center for Medicare and Medicaid Services spent nearly $32,000 per capita on stroke and heart failure, almost $29,000 (16).

Diseases that occur after metabolic disturbance are linked to a process that causes LDL oxidation. Typically, 60–70% of LDL moves back to the liver after circulation, and peripheral tissues take the remaining 30–40% take the remaining 30–40%. North America, Europe, and Asia have hosted the majority of the studies on MS (17). Because of this, little is known about the prevalence and risk factors of MS in the population of sub-Saharan Africa. According to the limited studies that have been done in sub-Saharan Africa, the incidence of MS is quickly catching up to that in affluent countries (18). It could result from harmful Western food and lifestyle changes, cigarette use, and anti-HIV medication usage in those regions (19). The prevalence of non-communicable diseases (NCDs) has recently grown in sub-Saharan nations like Ethiopia due to fast economic expansion, an aging population, and sedentary lifestyles (20).

Relationship between ox-LDL and metabolic disorders

Diabetes

T2DM is the most common type of diabetes mellitus. It is typically characterized by chronic hyperglycemia, hyperinsulinemia, dyslipidemia, and lipotoxicity, resulting in progressive deterioration of insulin secretion and insulin action (21). Hyperglycemia results from the overproduction of free radicals, which are linked to the development of diabetes (22). It has been found that insulin resistance plays a key role in the occurrence of T2DM. Risk factors often include hyperinsulinemia, decreased high-density lipoprotein (HDL) cholesterol, elevated triglyceride, and hypertension with insulin resistance (23). Adipocyte insulin resistance and inflammation have been identified as essential factors in the occurrence of T2DM (24). It is undeniable that insulin resistance is characterized by decreased peripheral glucose uptake (primarily in the muscles) and increased endogenous glucose production. In addition, it decreased peripheral glucose utilization and impaired beta-cell function (25).

Typically, in a way mentioned in Figure 1, glucose uptake, glucose moves inside the cells. But in insulin resistance, insulin receptors become resistant to insulin which ceases this mechanism, and insulin and glucose levels elevate in the bloodstream. Insulin resistance can disrupt glucose metabolism (26), resulting in chronic hyperglycemia, which causes OS and

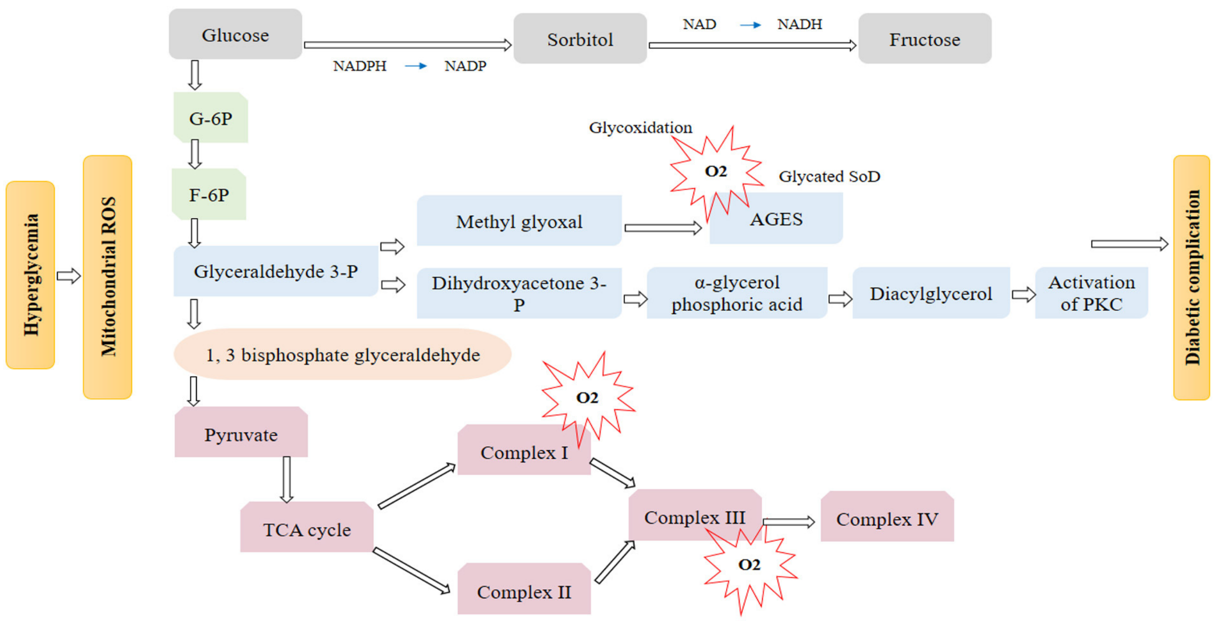

FIGURE 1
During high hyperglycemia, reactive oxygen species (ROS) is excessively increased in mitochondria. Three main pathway is responsible for hyperglycemia damage an activation of the polyols pathway, PKC pathway, and accumulation of AGES will prevent diabetic complications altogether.

inflammatory responses that cause cellular damage. LDL is exposed to high circulating glucose levels due to the high glucose concentration in the blood. This exposure changes the LDL to glycated LDL.

Diabetes is also caused by excessive reactive oxygen species (ROS) produced in obese people, which causes the proliferative arrest of pancreatic beta β-cells (27). Most -cells do not have the potential to re-enter the cell cycle or have a short cell cycle length. ROS plays a significant part in the dysregulation of pancreatic cell proliferation by changing the cell cycle regulators, contributing to the onset and progression of diabetes (27). Numerous studies have also shown a direct link between elevated OS in MS and nicotinamide adenine dinucleotide phosphate oxidase (NOX) activity (28, 29) (Figure 2).

Changing dietary intake from organic healthy foods to highly processed foods may lead to increased exposure to advanced glycated end products aged garlic extracts (AGEs) by a non-enzymatic chemical reaction called glycation (30). In industries, AGEs are used to improve flavor and color and increase the shelf life of food (30). On the other hand, increased exposure to these AGEs may lead to severe health disorders. There are two types of AGE: Serum endogenous advance glycated end products (sAGE) that form within the body during digestion, absorption, and metabolism (30). Foods are the exogenous AGEs also called dietary AGEs (dAGE's). Both endogenous and exogenous AGE's significantly contributed to the total body AGE pool (31).

Different Researches explain that older individuals have been exposed to both endogenous and exogenous AGE (32). It leads to the development and progression of severe health disorders (32). These age-related problems are mediated and associated with OS and inflammation (33). Increased daily intake of processed foods, deep-fried foods, and high fructose items may cause inflammation and disturbance of the immune system. The primary physiological effect of insulin on nutrient utilization and intermediary metabolism occurs in the postprandial state when variable increases in plasma glucose cause insulin secretion (34). It results in glucose clearance from plasma by stimulating its uptake, using skeletal muscle and adipose tissue, and attenuating hepatic glucose production by inhibiting hepatic gluconeogenesis and glycogenolysis (Figure 3).

Insulin resistance may affect lipid metabolism as much as glucose (38). Ox-LDL was significantly linked to insulin resistance in children, young adults, and the elderly. Diabetes patients had substantially higher ox-LDL levels than non-diabetics (38). Insulin's main effects also preserve skeletal muscle mass by inhibiting protein breakdown, translating specific protein groups, and inducing lipid accumulation in adipose tissue (39). However, insulin resistance can be any impairment of insulin action on target tissues (40).

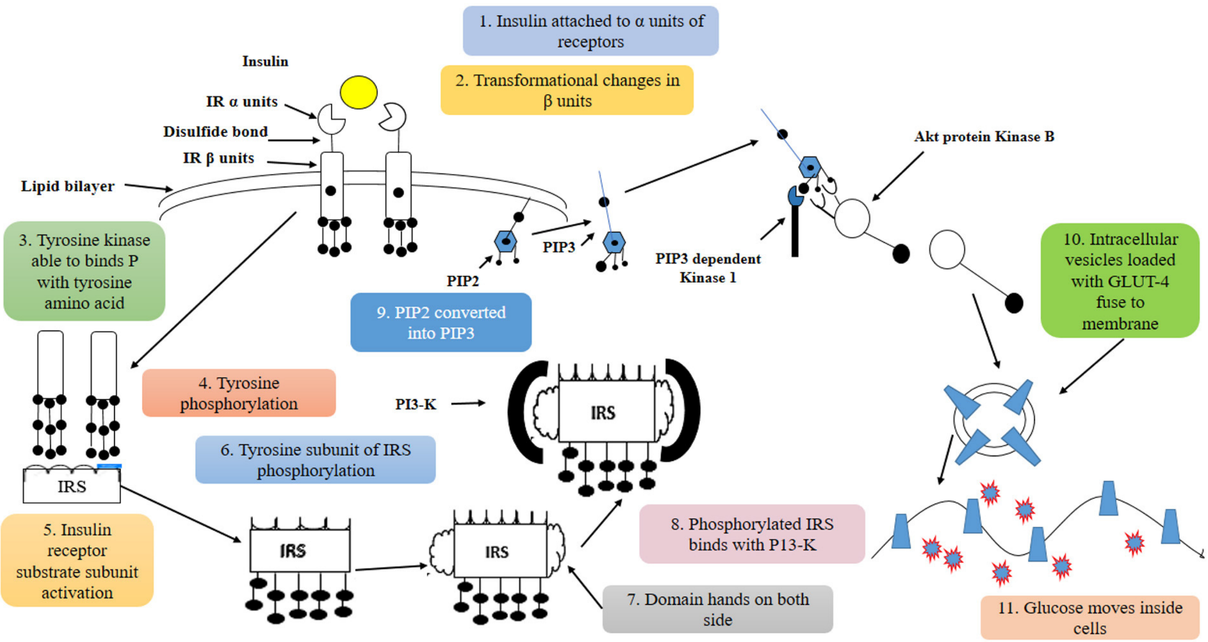

FIGURE 2
Insulin binds to insulin receptors α units which cause phosphorylation of insulin β units. After activating the insulin receptor substrate subunit, P13-K is attached to domain hands on both sides. This procedure activates Akt protein kinase B, which plays an important role in transferring glucose inside the cells.

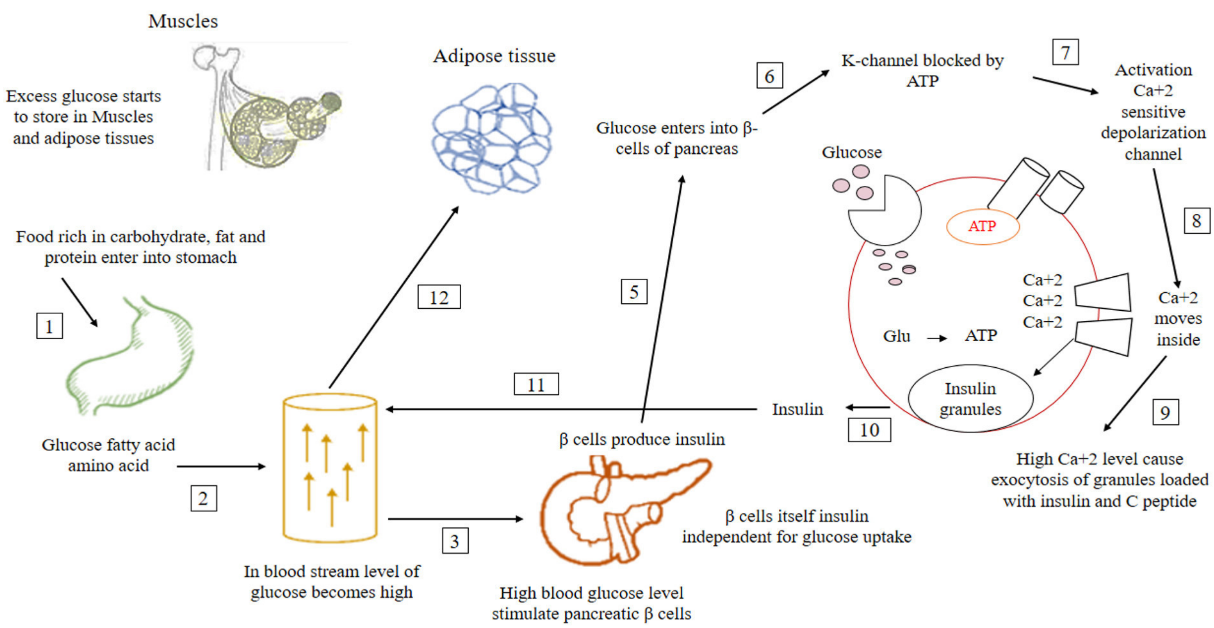

FIGURE 3
In normal conditions, after taking food, the glucose level of the blood increases. High glucose levels stimulate pancreatic β cells responsible for insulin production. After the activation of β cells, insulin level increases in the blood, which lowers glucose level to the normal range. When this insulin production is not enough for glucose utilization, glucose remains constant in the blood (35–37).

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OS in metabolic disorder leading to obesity

OS is a double-edged sword since it can cause and result in obesity. Numerous epidemiological, animal, and clinical investigations have shown that obesity and redox change are related (41). Increased OS can result from several variables, such as high-fat, high-carbohydrate diets, and persistent undernutrition, by activating intracellular pathways such as NOX, oxidative phosphorylation in mitochondria, glycoxidation, protein kinase C (PKC), and the polyol pathway (41). OS and obesity are related through mutual support pathways (42). In addition to causing a persistent chronic inflammatory state by excessive ROS formation due to a high-fat, high-carbohydrate diet and inhibited antioxidant system, obesity can also produce systemic OS through NOX activation (27). Even though it is difficult to pinpoint which comes first, inflammation and OS coexist in obesity (27). The overproduction of ROS may be further exacerbated by the redox-sensitive transcription factors, including NF-kB and activator protein (AP)-1, which are activated by ROS and produce many pro-inflammatory cytokines (27). It causes a cycle that breeds a variety of illnesses, including insulin resistance, T2DM, atherosclerosis, and cancer, all referred to as MS (27, 43).

Atherosclerosis

Several genetic and environmental factors lead to CVDs. Oxidation of LDL is the main factor that leads to subclinical CVDs by initiating atherogenic events. These events cause the formation of mature atherosclerotic plaque. Atherosclerosis is a disease in which blood delivery and flow reduce all across the body due to the hardening and thickening of blood vessels (44).

The oxidation of LDL also aids the formation of atherosclerotic plaques. Atherosclerosis is a condition of developing complicated atherosclerotic plaques and causes the hardening and narrowing of the arterial wall (45). The Greek term atherosclerosis consists of two parts, the first one is Atherosis and the second one is sclerosis. Atherosis means fat accumulation goes along with several macrophages, and sclerosis is defined as a fibrosis layer consisting of smooth muscle cells, connective tissues, and leukocytes (46).

LDL does not directly promote atherosclerosis, but its oxidative modification in intima can increase foam cell formation at the lesion site and atherosclerotic plaque formation (47). For a clear understanding of how ox-LDL leads to atherosclerosis, there is a need to explain the whole mechanism of atherosclerosis. The atherosclerosis process includes three main steps: 1. Formation of fatty streaks, 2. Formation of atheroma, and 3. Formation of atherosclerotic plaque.

Fatty strips formation

For a clear understanding of how ox-LDL leads to atherosclerosis, there is a need to explain the whole mechanism of atherosclerosis (48). The atherosclerosis process includes: (a): In the first step, due to elevated level of plasma LDL cholesterol, LPL-C entered endothelial cells by endocytosis. Because of the high level of plasma LDL, extracellular proteoglycans increase (49). LDL has a great affinity with this extracellular component, so it gets trapped at the lesion side in the intima wall of arteries. So, the Concentration and period of stay in intima increased (50). These factors result in spontaneous oxidative modification of trapped LDL. (b) In a second step, ox-LDL functions as an antigen for T-cells and activates T-cells, so accordingly secrete cytokines that initiate endothelial, smooth muscle cells and macrophages SMS in further steps. (c): In the third step, activated or altered endothelial cells generate adhesion molecules on leukocytes (51). Adhesion molecules have specific receptors expressing smooth muscle cells and vascular endothelial cells on specific leukocytes. In the expression of adhesion receptors, transcription factor NF-αβ is activated by pro-inflammatory binding cytokines to their receptors on the endothelial surface and performed transcription (52). These adhesion molecules play a vital role in chemokines/cytokine production and release, which is critical in the activation and release of leukocytes. Furthermore, migration of endothelial and smooth muscle cells (SMC) accurse due to specific chemokines. From various studies, it has been demonstrated that adhesion molecules are unregulated by ox-LDL. (d): In the fourth step, phagocytes differentiate into macrophages after insertion into the intima. Macrophages carry out uptake and acquisition of ox-LDL with the action of their scavenger receptors. They will convert to yellowish foam cells—cytokines and ox-LDL increase the expression of these receptors when monocytes differentiate into macrophages (51). Ox-LDL ligand surface is phospholipids that start their absorption to receptors which will be oxidized at no two locations and result in the formation of aldehydes that have the power to attack Apo lysine residues. These yellow foam cells accumulated on the walls of arteries, and lipid strips formed. Monocytes can also produce cytotoxic substances, leading to more LDL oxidation and damage (51).

Formation of atheroma

Endothelial cells secret small peptides such as cytokines and growth factors like interleukin 1 (IL-1) and TNF causing smooth muscle cell migration and synthesized extracellular matrix. It forms the fibrous cap of collagen-rich fiber tissues, SMC, macrophages, and T-lymphocytes (46).

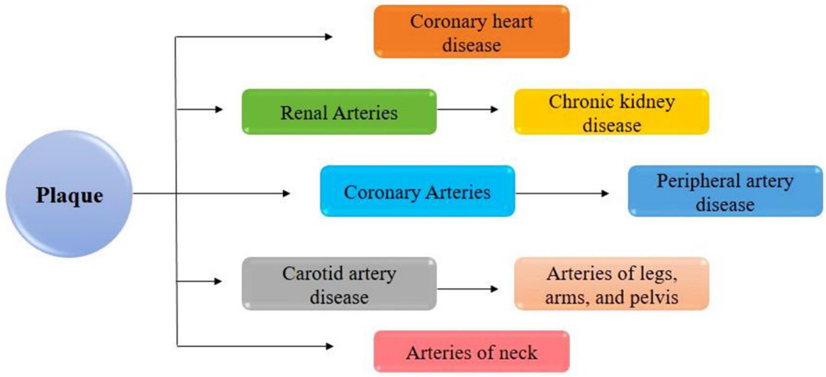

FIGURE 4
LDL is not directly promoting atherosclerosis, but its oxidative modification in intima can lead to increases in foam cell formation at the lesion site and atherosclerotic plaque formation (53).

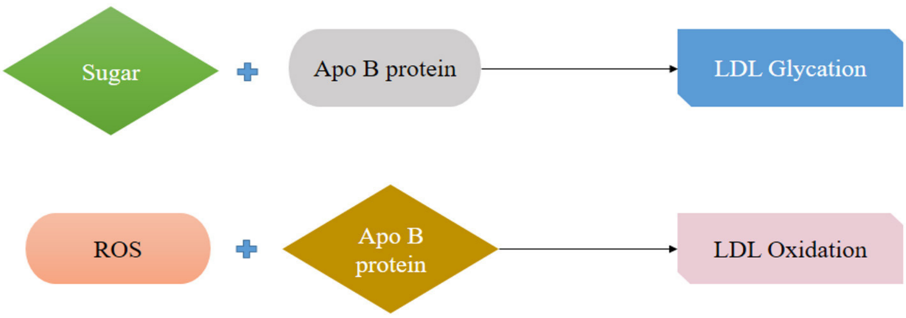

FIGURE 5
Attachment of sugar with protein strand (Apo B) is called LDL glycation, modification of Apo B by Free radicals known as LDL oxidation.

Formation of atherosclerosis plaque

All the above factors formed mature atherosclerotic plaque that further obstructs arteries' blood flow. So, ox-LDL can be diligently involved in the atherosclerotic process by different mechanisms, including the activation of t-cells. Endothelial cell (EC) activation and dysfunction, activation of macrophage (Figure 4). By up-regulated adhesion molecules, foam cell formation by increasing the expression of scavenger receptors of macrophages and by proliferation and migration of vascular smooth muscle cells (VSMC) (46, 54, 55).

Dietary management for a reduction in oxidation and glycation of LDL

Taking foods containing complex carbohydrates like vegetables, fruits, cereals, and dairy products has a low concentration of AGEs (56). Avoid snacks, biscuits, and other processed foods as they contain high levels of AGEs. The cooking method and heating duration played an essential role in the increased production of AGEs (56). Deep frying increased the concentration of glycated products. Take adequate vitamin C, B, and phytonutrients (56) (Figure 5).

Dietary approaches to inhibit LDL oxidation and glycation

Mono-unsaturated fatty acid

A study was established to compare the effect of high monounsaturated fatty acid and a high carbohydrate diet on LDL oxidation (57). To reach the result, twenty men and women were taken with diabetes mellitus and a mean age of 61. They were given an isocaloric diet with carbohydrates (28% energy) and monounsaturated fatty acid (MUFA) (40% energy) for 6 weeks. After 6 weeks, LDL susceptibility to oxidation, body weight, glycemic control, and lipid profile were measured. It was concluded that both high carbohydrate and high MUFA natural food-based diets have a similar effect on LDL oxidative resistance and overall metabolic control in patients with diabetes mellitus (58). Body weight, total cholesterol and triglycerides were also the same after the two diets. Still, the only difference was that the high monounsaturated fatty acid diet lowered the very-low-density lipoprotein (VLDL) by 35% compared to a high carbohydrate diet. MUFA was also a good alternative to a high carbohydrate diet for T2DM (Figure 6).

High fat diet

A study showed the effect of a high-fat diet in lowering plasma triglycerides and VLDL concentration in patients with diabetes mellitus (59). This effect was due to increased lipolysis activity or increased clearance of triglyceride-rich lipoprotein (60). It was seen that HDL concentration also increased after the consumption of a high monounsaturated diet as compared to a high carbohydrate diet (61). The net increase was 0.05 mmol and reduced the susceptibility of LDL oxidation (61). According to the study, the subjects fed a diet rich in oleic acid were less susceptible to oxidation (62). Diet rich in monounsaturated acid has 27% more α-tocopherol than compared to a diet rich in carbohydrates (62). It has a protective role against LDL oxidation due to its antioxidant properties.

Vitamin E

Diabetes lowers antioxidant vitamin levels, making lipids more vulnerable to oxidative assault. Lipid-soluble antioxidants carry LDL like vitamin E and carotenoids (including b-carotene and lycopene) that shield it from oxidation (63). A significant water-soluble chain-breaking antioxidant, vitamin C (ascorbate), works by rebuilding a-tocopherol from its oxidized radical state (64). Diabetes patients have been shown to have lower vitamin E and ascorbate (65).

It may be due to the antioxidant property of Vitamin E, which reduces the number of oxidants and free radicals and have a protective role in lipid oxidation (66). With the admission of vitamin E, paraoxonase 1 protein has expanded in the blood, showing a decline in OS and protecting the lipids from oxidation (67).

Polyphenols

Polyphenols are the primary antioxidants in the human diet (68–70). They have antioxidant and anti-inflammatory properties and have a protective role against chronic health problems that involve inflammation (71–73). Culinary herbs and spices have a higher concentration of phenolic compounds and low caloric content, which is advantageous in diabetes mellitus (74, 75). One of the results of raised blood glucose is an expansion in the nonenzymatic glycation of proteins. Evidence showed that the extract of these herbs and spices might block the formation of advanced glycated end products (AGEs) (76). *In vitro* experiment showed that 50% ethanolic extracts of these herbs and spices inhibit fructose-mediated protein glycation. Extracts of cinnamon and ground Jamaican allspice are the most effective inhibitors of glycation (77, 78).

Histidine and carnosine

Histidine and carnosine are synthesized in the liver, skeletal muscle, and brain. These compounds are known for their antioxidant properties, such as scavenging free radicals binding the metal ions and inhibiting glycation (79). LDL oxidation and glycation result from high blood glucose (80). That cause vascular damage and further complication. Research suggests that histidine and carnosine might protect against LDL oxidation and glycation (81). Because they are amino acid-base compounds with a higher affinity for water-soluble molecules, they may compete for glucose with the apo-B part of the LDL molecule, allowing them to postpone the glycation process between glucose and the LDL protein part (82).

After the estimated time, ingestion of histidine and carnosine at a ratio of 1 g/L in diabetic mice significantly reduced blood glucose and fibronectin levels (81, 83). These agents showed a dose-dependent effect in suppressing malondialdehyde formation and glycation (81). Treatments with 1 g/l histidine and carnosine significantly enhanced glutathione peroxidase activity (81). In diabetic mice, consumption of histidine or carnosine greatly reduced the activity of interleukin (IL) 6 and tumor necrosis factor (TNF) alpha (84).

Garlic extract

In different studies, aged garlic extract inhibited LDL oxidation and reduced oxidized-induced cell injury (85, 86). The antioxidant effects of AGE were investigated further using bovine pulmonary artery endothelial cells (PAEC) and murine macrophages (86). Lactate dehydrogenase (LDH) release and intracellular glutathione (GSH) levels were measured as indicators of membrane injury. Ox-LDL increased LDH release while depleting GSH. These changes were prevented by pretreatment with AGE (86).

L-carnitine

L-carnitine protects against CVD by increasing HDL cholesterol, inhibiting LDL cholesterol oxidation, and neutralizing the atherogenic effects of ox-LDL cholesterol (87). Reduced concentrations of TBARS (Thiobarbituric acid reactive substances) and conjugated dienes, which are indices of lipid peroxidation, in the blood of diabetic hyperlipidemia patients (87). These lower concentrations could be attributed to a decrease in or increase in the use of antioxidant mechanisms (87). Changes in the composition of LDL cholesterol may result in conformational changes, resulting in a different exposure of fatty acids to oxygen-free radicals and changes in the rate of lipid peroxidation (88).

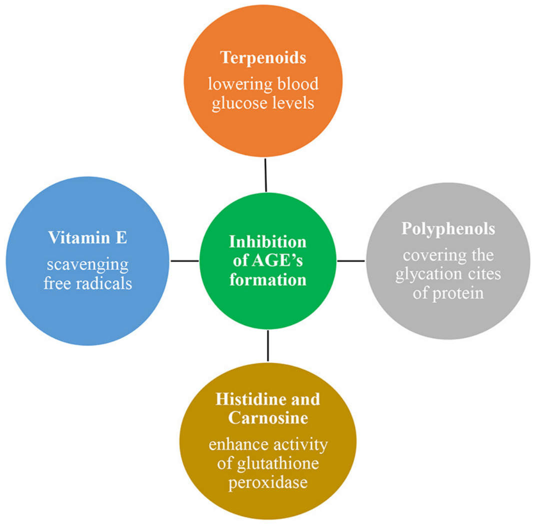

FIGURE 6
Vitamin E, Polyphenols, Terpenoids, Histidine, and carnosine have antioxidant and anti-inflammatory properties and protective roles against LDL oxidation and glycation. They protect by scavenging free radicals by covering the glycation sites of protein. And lowers the blood glucose levels.

Novel strategies

New combination approaches have been used to target glycolysis (through targeting PKM2 or pyruvate dehydrogenase kinase) and increasing oxidative phosphorylation, resulting in increased OS in cancer cells (89, 90). Different studies found that inhibiting de-novo lipogenesis in prostate cancer cells with soraphen A (an inhibitor of acetyl Co- carboxylase) causes an increase in polyunsaturated fatty acids, OS, and greater sensitivity to chemotherapeutic treatments (91). Overall, OS is integral to carcinogenesis and cancer cell metabolism and presents novel treatment possibilities (91). Phytochemicals like green tea component epigallocatechin-3 gallate and turmeric component curcumin have been shown to reduce obesity-associated polyp formation in animal models by inhibiting PI3K/Akt and MAPK signal pathways and have been suggested as a prevention strategy for obesity-associated colon cancer (92). Combination therapies target glycolysis (by targeting PKM2 or pyruvate dehydrogenase kinase) and encourage oxidative phosphorylation, leading to increased OS (93). Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with MS have also been studied in randomized control trials (94).

Lifestyle modification

Lifestyle factors such as smoking, drinking alcohol, eating a proper or improper diet, exercising, and being untrained all contribute to OS (95). According to different studies, ROS exists in muscles and controls muscle function (96). ROS are continually produced at low levels by skeletal muscle fibers, and these levels rise during muscular contraction. They are implicated in skeletal muscle exhaustion during intense exercise and have several direct and indirect impacts on muscle function (contractility, excitability, metabolism, and calcium homeostasis) (96).

Restoring the body's redox equilibrium is one of the most acceptable ways to eliminate harmful OS (97). The objective may be to regain a healthy BMI through physical exercise and a low-fat, low-carbohydrate diet rich in antioxidants (98). A clinical investigation found that weight loss reduces signs of OS and boosts the antioxidant system, lowering the risk of CVDs linked with obesity (99). Natural fruits (100), green vegetables (101), whole grains (102), legumes (101), fish (103), olive oil (104), and probiotics (105), which are high in MUFA and polyunsaturated fatty acids (PUFA), vitamin C, vitamin E, and phytochemicals, aid in weight management and reduce the risk of metabolic diseases *via* a variety of mechanisms including cell signaling, altered gene expression, and decreased OS (101). Physical activity and exercise improve the body's antioxidant system, which aids in the management of OS by scavenging harmful free radicals, and alters cell-signaling pathways, which activate detoxification enzymes, reduce inflammation, promote normal cell cycle, inhibit proliferation, induce apoptosis, and prevent tumor invasion and angiogenesis (101).

Conclusion

OS is linked to all modern diseases. Diabetes, CVDs, and cancer are the top causes of death worldwide. These disorders are brought on by OS, which causes inflammation. LDL is oxidized, forming AGEs and ox-LDL end products, damaging the cellular mechanism and disturbing function. This damage results in the development of diseases. Dietary oxidation is a significant cause of ox-LDL and AGEs, which can be addressed through appropriate diet management and consumption of suitable phytonutrients. Since OS has become a major factor in chronic metabolic diseases, it is crucial to: (i) further understand the mechanisms that disturb the healthy balance between oxidative and antioxidative processes; (ii) incorporate various nutritional antioxidants into current therapies, including those that can neutralize OS, such as flavonoids, arginine, vitamin C, vitamin E, carotenoids, resveratrol, and selenium; and (iii) prevent mitochondrial dysfunction from boosting defenses against OS.

Author contributions

Conceptualization: MM, NA, MA, and ARa. Writing—original draft preparation: ZA, AK, IM, DM, GG, ARa, AA, and MM. Writing—review and editing: MM, NA, MA, and ARu. Revision of article: VC, MM, and AA. Supervision: MM and ARu. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Haile K, Timerga A. Dyslipidemia and its associated risk factors among adult type-2 diabetic patients at Jimma University Medical Center, Jimma, Southwest Ethiopia. *Diabetes Metab Syndr Obes.* (2020) 13:4589. doi: 10.2147/DMSO.S283171

2. Shah IU, Sameen A, Manzoor MF, Ahmed Z, Gao J, Farooq U, et al. Association of dietary calcium, magnesium, and vitamin D with type 2 diabetes among US adults: National health and nutrition examination survey 2007–2014—A cross-sectional study. *Food Sci Nutr.* (2021) 9:1480–90. doi: 10.1002/fsn3.2118

3. Wang HH, Lee DK, Liu M, Portincasa P, Wang DQ-H. Novel insights into the pathogenesis and management of the metabolic syndrome. *Pediatr Gastroenterol Hepatol Nutr.* (2020) 23:189. doi: 10.5223/pghn.2020.23.3.189

4. Montoya-Estrada A, Veruete-Bedolla DB, Romo-Yañez J, Ortiz-Luna GF, Arellano-Eguiluz A, Najéra N, et al. Markers of oxidative stress in postmenopausal women with metabolic syndrome. *J Obs Gynaecol.* (2022) 1–6. doi: 10.1080/01443615.2022.2062223

5. Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: diversity, patterns of recognition, and pathophysiology. *Antioxid Redox Signal.* (2010) 13:39–75. doi: 10.1089/ars.2009.2733

6. Julibert A, Bibiloni MDM, Mateos D, Angullo E, Tur JA. Dietary fat intake and metabolic syndrome in older adults. *Nutrients.* (2019) 11:1901. doi: 10.3390/nu11081901

7. World Health Organization. *The Top 10 Causes of Death* .(2020). World Health Organization. Available online at: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (accessed May 9, 2022).

8. British Heart Foundation. *Heart Statistics.* (2022). British Heart Foundation. Available online at: https://www.bhf.org.uk/what-we-do/our-research/heart-statistics (accessed May 23, 2022).

9. Poznyak A, Grechko AV, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN. The diabetes mellitus–atherosclerosis connection: the role of lipid and glucose metabolism and chronic inflammation. *Int J Mol Sci.* (2020) 21:1835. doi: 10.3390/ijms21051835

10. Misra S, Shishehbor M, Takahashi E, Aronow H, Brewster L, Bunte M, et al. Council on clinical cardiology; council on cardiovascular and stroke nursing, perfusion assessment in critical limb ischemia: principles for understanding and the development of evidence and evaluation of devices: a scientific statement from the American Heart Association. *Circulation.* (2019) 140:e657–72. doi: 10.1161/CIR.0000000000000708

11. Rehman T, Shabbir MA, Inam-Ur-Raheem M, Manzoor MF, Ahmad N, Liu ZW, et al. Cysteine and homocysteine as biomarker of various diseases. *Food Sci Nutr.* (2020) 8:4696–707. doi: 10.1002/fsn3.1818

12. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol.* (2019) 11:45.

13. Shah AA, Rehman A, Haider AH, Sayani R, Sayyed RH, Ali K, et al. Angiographic embolization for major trauma in a low-middle income healthcare setting–A retrospective review. *Int J Surg.* (2015) 18:34–40. doi: 10.1016/j.ijsu.2015.03.023

14. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic. epidemiologic, and clinical studies A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J.* (2017) 38:2459–72. doi: 10.1093/eurheartj/ehx144

15. News C. *Heart Disease Could Cost U.S. $1 Trillion a Year by 2035.* (2017). Available online at: https://www.cbsnews.com/news/heart-disease-could-cost-us-1-trillion-per-year-by-2035/ (accessed July 28 2022).

16. American Heart Association. *Investing in Heart Disease and Stroke Research.* (2019). American Heart Association. Available online at: https://www.heart.org/-/media/Files/About-Us/Policy-Research/Fact-Sheets/Public-Health-Advocacy-and-Research/Investing-in-Our-Hearts--NIH-Fact-Sheet.pdf (accessed July 28, 2022).

17. Khalili M, Shuhart MC, Lombardero M, Feld JJ, Kleiner DE, Chung RT, et al. Relationship between metabolic syndrome, alanine aminotransferase levels, and liver disease severity in a multiethnic north American cohort with chronic hepatitis B. *Diabetes Care.* (2018) 41:1251–9. doi: 10.2337/dc18-0040

18. Anyanwu BO, Ezejiofor AN, Igweze ZN, Orisakwe OE. Heavy metal mixture exposure and effects in developing nations: an update. *Toxics.* (2018) 6:65. doi: 10.3390/toxics6040065

19. Kamari Y, Grossman E, Oron-Herman M, Peleg E, Shabtay Z, Shamiss A, et al. Metabolic stress with a high carbohydrate diet increases adiponectin levels. *Horm Metabol Res.* (2007) 39:384–8. doi: 10.1055/s-2007-976534

20. Okafor CI. The metabolic syndrome in Africa: current trends. *Indian J Endocrinol Metab.* (2012) 16:56. doi: 10.4103/2230-8210.91191

21. Maciejczyk M, Żebrowska E, Chabowski A. Insulin resistance and oxidative stress in the brain: whatŠs new? *Int J Mol Sci.* (2019) 20:874.

22. Tuleab SF. Glutathione, Vitamin C, Malonodialdehyde oxidized low-density lipoprotein and lipid profile levels in type 2 diabetic Iraqi Males. *Al-Nahrain J Sci.* (2016) 19:48–55. doi: 10.22401/JNUS.19.1.06

23. Reusch JE. Current concepts in insulin resistance, type 2 diabetes mellitus, and the metabolic syndrome. *Am J Cardiol.* (2002) 90:19–26. doi: 10.1016/S0002-9149(02)02555-9

24. Leahy JL. Pathogenesis of type 2 diabetes mellitus. *Arch Med Res.* (2005) 36:197–209. doi: 10.1016/j.arcmed.2005.01.003

25. Behrens G, Dejam A, Schmidt H, Balks H-J, Brabant G, Körner T, et al. Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. *Aids.* (1999) 13:F63–70. doi: 10.1097/00002030-199907090-00001

26. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. *Nat Med.* (2017) 23:804–14. doi: 10.1038/nm.4350

27. Rani V, Deep G, Singh RK, Palle K, Yadav UC. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sci.* (2016) 148:183–93. doi: 10.1016/j.lfs.2016.02.002

28. Elnakish MT, Hassanain HH, Janssen PM, Angelos MG, Khan M. Emerging role of oxidative stress in metabolic syndrome and cardiovascular diseases: important role of Rac/NADPH oxidase. *J Pathol.* (2013) 231:290–300. doi: 10.1002/path.4255

29. Schiffer TA, Lundberg JO, Weitzberg E, Carlström M. Modulation of mitochondria and NADPH oxidase function by the nitrate-nitrite-NO pathway in metabolic disease with focus on type 2 diabetes. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease.* (2020) 1866:165811. doi: 10.1016/j.bbadis.2020.165811

30. Fallavena LP, Rodrigues NP, Marczak LDF, Mercali GD. Formation of advanced glycation end products by novel food processing technologies: a review. *Food Chem.* (2022) 133338. doi: 10.1016/j.foodchem.2022.133338

31. Almajwal AM, Alam I, Abulmeaty M, Razak S, Pawelec G, Alam W. Intake of dietary advanced glycation end products influences inflammatory markers, immune phenotypes, and antiradical capacity of healthy elderly in a little-studied population. *Food Sci Nutr.* (2020) 8:1046–57. doi: 10.1002/fsn3.1389

32. Ávila-Escalante ML, Coop-Gamas F, Cervantes-Rodríguez M, Méndez-Iturbide D, Aranda-González II. The effect of diet on oxidative stress and metabolic diseases—Clinically controlled trials. *J Food Biochem.* (2020) 44:e13191. doi: 10.1111/jfbc.13191

33. Brunt VE, Gioscia-Ryan RA, Richey JJ, Zigler MC, Cuevas LM, Gonzalez A, et al. Suppression of the gut microbiome ameliorates age-related arterial dysfunction and oxidative stress in mice. *J Physiol.* (2019) 597:2361–78. doi: 10.1113/JP277336

34. Chen M, Nagase M, Fujita T, Narumiya S, Masaki T, Sawamura T. Diabetes enhances lectin-like oxidized LDL receptor-1 (LOX-1) expression in the vascular endothelium: possible role of LOX-1 ligand and AGE. *Biochem Biophys Res Commun.* (2001) 287:962–8. doi: 10.1006/bbrc.2001.5674

35. Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. Sugar absorption in the intestine: the role of GLUT2. *Annu Rev Nutr.* (2008) 28:35–54. doi: 10.1146/annurev.nutr.28.061807.155518

36. Cura AJ, Carruthers A. The role of monosaccharide transport proteins in carbohydrate assimilation, distribution, metabolism and homeostasis. *Compr Physiol.* (2012) 2:863. doi: 10.1002/cphy.c110024

37. Ebert K, Ludwig M, Geillinger KE, Schoberth GC, Essenwanger J, Stolz J, et al. Reassessment of GLUT7 and GLUT9 as putative fructose and glucose transporters. *J Membr Biol.* (2017) 250:171–82. doi: 10.1007/s00232-016-9945-7

38. Lee E, Jung S-R, Lee S-Y, Lee N-K, Paik H-D, Lim S-I. Lactobacillus plantarum strain Ln4 attenuates diet-induced obesity, insulin resistance, and changes in hepatic mRNA levels associated with glucose and lipid metabolism. *Nutrients.* (2018) 10:643. doi: 10.3390/nu10050643

39. Borg ML, Massart J, De Castro Barbosa T, Archilla-Ortega A, Smith JA, Lanner JT, et al. Modified UCN2 peptide treatment improves skeletal muscle mass and function in mouse models of obesity-induced insulin resistance. *J Cachexia Sarcopenia Muscle.* (2021) 12:1232–48. doi: 10.1002/jcsm.12746

40. Barazzoni R, Gortan Cappellari G, Ragni M, Nisoli E. Insulin resistance in obesity: an overview of fundamental alterations. *Eat Weight Disord.* (2018) 23:149–57. doi: 10.1007/s40519-018-0481-6

41. Korac B, Kalezic A, Pekovic-Vaughan V, Korac A, Jankovic A. Redox changes in obesity, metabolic syndrome, and diabetes. *Redox Biol.* (2021) 42:101887. doi: 10.1016/j.redox.2021.101887

42. Marseglia L, Manti S, D'angelo G, Nicotera A, Parisi E, Di Rosa G, et al. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci.* (2014) 16:378–400. doi: 10.3390/ijms16010378

43. Ali A, Manzoor MF, Ahmad N, Aadil RM, Qin H, Siddique R, et al. The Burden of Cancer, Government Strategic Policies, and Challenges in Pakistan: a comprehensive review. *Front Nutr.* (2022) 1553. doi: 10.3389/fnut.2022.940514

44. Cheng MJ, Mitra R, Okorafor CC, Nersesyan AA, Harding IC, Bal NN, et al. Targeted intravenous nanoparticle delivery: role of flow and endothelial glycocalyx integrity. *Ann Biomed Eng.* (2020) 48:1941–54. doi: 10.1007/s10439-020-02474-4

45. Lee Y-T, Hsu C-C, Lin M-H, Liu K-S, Yin M-C. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Eur J Pharmacol.* (2005) 513:145–50. doi: 10.1016/j.ejphar.2005.02.010

46. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med.* (2014) 5:927.

47. Linton MF, Yancey PGDavies SS, Jerome WGLinton EF, Song WL, et al. *The Role of Lipids and Lipoproteins in Atherosclerosis*. Endotext (2019).

48. Chen T, Huang W, Qian J, Luo W, Shan P, Cai Y, et al. Macrophage-derived myeloid differentiation protein 2 plays an essential role in ox-LDL-induced inflammation and atherosclerosis. *EBioMed.* (2020) 53:102706. doi: 10.1016/j.ebiom.2020.102706

49. Scuruchi M, Potì F, Rodríguez-Carrio J, Campo GM, Mandraffino G. Biglycan and atherosclerosis: lessons from high cardiovascular risk conditions. *Biochimica et Biophysica Acta Molecular and Cell Biology of Lipids.* (2020) 1865:158545. doi: 10.1016/j.bbalip.2019.158545

50. Mahjoubin-Tehran M, Kovanen PT, Xu S, Jamialahmadi T, Sahebkar A. Cyclodextrins: Potential therapeutics against atherosclerosis. *Pharmacol Therapeut.* (2020) 214:107620. doi: 10.1016/j.pharmthera.2020.107620

51. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, et al. Pathophysiology of atherosclerosis. *Int J Mol Sci.* (2022) 23:3346. doi: 10.3390/ijms23063346

52. Zhou W, Yang L, Nie L, Lin H. Unraveling the molecular mechanisms between inflammation and tumor angiogenesis. *Am J Cancer Res.* (2021) 11:301.

53. Linton MF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. *The Role of Lipids and Lipoproteins in Atherosclerosis*. Endotext (2019).

54. Ho RC, Davy K, Davy B, Melby CL. Whole-body insulin sensitivity, low-density lipoprotein (LDL) particle size, and oxidized LDL in overweight, nondiabetic men. *Metabol Clin Exp.* (2002) 51:1478–83. doi: 10.1053/meta.2002.35577

55. Di Pietro N, Formoso G, Pandolfi A. Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. *Vascul Pharmacol.* (2016) 84:1–7. doi: 10.1016/j.vph.2016.05.013

56. Willett WC, Stampfer MJ. Rebuilding the food pyramid. *Sci Am.* (2003) 288:64–71. doi: 10.1038/scientificamerican0103-64

57. Colquhoun DM, Moores D, Somerset SM, Humphries JA. Comparison of the effects on lipoproteins and apolipoproteins of a diet high in monounsaturated fatty acids, enriched with avocado, and a high-carbohydrate diet. *Am J Clin Nutr.* (1992) 56:671–7. doi: 10.1093/ajcn/56.4.671

58. Mirabelli M, Chiefari E, Arcidiacono B, Corigliano DM, Brunetti FS, Maggisano V, et al. Mediterranean diet nutrients to turn the tide against insulin resistance and related diseases. *Nutrients.* (2020) 12:1066. doi: 10.3390/nu12041066

59. Magalhães DA, Kume WT, Correia FS, Queiroz TS, Allebrandt EW, Santos MP, et al. High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. *Anais da Academia Brasileira de Ciências.* (2019) 91:e20180314. doi: 10.1590/0001-3765201920180314

60. Lim S, Kim YJ, Khang AR, Eckel RH. Postprandial dyslipidemia after a standardized high-fat meal in BMI-matched healthy individuals, and in subjects with prediabetes or type 2 diabetes. *Clin Nutr.* (2021) 40:5538–46. doi: 10.1016/j.clnu.2021.09.004

61. Yki-Järvinen H, Luukkonen PK, Hodson L, Moore JB. Dietary carbohydrates and fats in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol.* (2021) 18:770–86. doi: 10.1038/s41575-021-00472-y

62. Tutunchi H, Ostadrahimi A, Saghafi-Asl M. The effects of diets enriched in monounsaturated oleic acid on the management and prevention of obesity: a systematic review of human intervention studies. *Adv Nutr.* (2020) 11:864–77. doi: 10.1093/advances/nmaa013

63. Blasa M, Gennari L, Angelino D, Ninfali P. Fruit and vegetable antioxidants in health. In: *Bioactive Foods in Promoting Health*. Amsterdam: Elsevier (2010). p. 37–58.

64. Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys.* (1993) 300:535–43. doi: 10.1006/abbi.1993.1074

65. Ibuki FK, Bergamaschi CT, Da Silva Pedrosa M, Nogueira FN. Effect of vitamin C and E on oxidative stress and antioxidant system in

the salivary glands of STZ-induced diabetic rats. *Arch Oral Biol.* (2020) 116:104765. doi: 10.1016/j.archoralbio.2020.104765

66. Babar Q, Ali A, Saeed A, Tahir MF. *Novel Treatment Strategy against COVID-19 through Anti-Inflammatory, Antioxidant and Immunostimulatory Properties of the B Vitamin Complex.* (2021).

67. Maes M, Bonifacio KL, Morelli NR, Vargas HO, Moreira EG, St Stoyanov D, et al. Generalized anxiety disorder (GAD) and comorbid major depression with GAD are characterized by enhanced nitro-oxidative stress, increased lipid peroxidation, and lowered lipid-associated antioxidant defenses. *Neurotox Res.* (2018) 34:489–510. doi: 10.1007/s12640-018-9906-2

68. Manzoor MF, Ahmad N, Ahmed Z, Siddique R, Zeng XA, Rahaman A, et al. Novel extraction techniques and pharmaceutical activities of luteolin and its derivatives. *J Food Biochem.* (2019) 43:e12974. doi: 10.1111/jfbc.12974

69. Ahmed N, Ali A, Riaz S, Ahmad A, Aqib M. *Vegetable Proteins: Nutritional Value, Sustainability, and Future Perspectives.* (2021).

70. Manzoor MF, Hussain A, Sameen A, Sahar A, Khan S, Siddique R, et al. Novel extraction, rapid assessment and bioavailability improvement of quercetin: A review. *Ultrason Sonochem.* (2021) 78:105686. doi: 10.1016/j.ultsonch.2021.105686

71. Ali A, Ain Q, Saeed A, Khalid W, Ahmed M, Bostani A. *Bio-Molecular Characteristics of Whey Proteins with Relation to Inflammation*. (2021).

72. Manzoor MF, Hussain A, Tazeddinova D, Abylgazinova A, Xu B. Assessing the nutritional-value-based therapeutic potentials and non-destructive approaches for mulberry fruit assessment: an overview. *Comput Intell Neurosci.* (2022) 2022:6531483. doi: 10.1155/2022/6531483

73. Wang L, Zheng W, Yang J, Ali A, Qin H. Mechanism of Astragalus membranaceus alleviating acquired hyperlipidemia induced by high-fat diet through regulating lipid metabolism. *Nutrients.* (2022) 14:954. doi: 10.3390/nu14050954

74. Pereira A, Fraga-Corral M, García-Oliveira P, Jimenez-Lopez C, Lourenço-Lopes C, Carpena M, et al. Culinary and nutritional value of edible wild plants from northern Spain rich in phenolic compounds with potential health benefits. *Food Funct.* (2020) 11:8493–515. doi: 10.1039/D0FO02147D

75. Anwar H, Rasul A, Iqbal J, Ahmad N, Imran A, Malik SA, et al. Dietary biomolecules as promising regenerative agents for peripheral nerve injury: an emerging nutraceutical-based therapeutic approach. *J Food Biochem.* (2021) 45:e13989. doi: 10.1111/jfbc.13989

76. Starowicz M, Zieliński H. Inhibition of advanced glycation end-product formation by high antioxidant-leveled spices commonly used in European cuisine. *Antioxidants.* (2019) 8:100. doi: 10.3390/antiox8040100

77. Dearlove RP, Greenspan P, Hartle DK, Swanson RB, Hargrove JL. Inhibition of protein glycation by extracts of culinary herbs and spices. *J Med Food.* (2008) 11:275–81. doi: 10.1089/jmf.2007.536

78. Ahmed M, Ali A, Sarfraz A, Hong Q, Boran H. Effect of freeze-drying on apple pomace and pomegranate peel powders used as a source of bioactive ingredients for the development of functional yogurt. *J Food Qual.* (2022). doi: 10.1155/2022/3327401

79. Khalid W, Ali A, Arshad MS, Afzal F, Akram R, Siddeeg A, et al. Nutrients and bioactive compounds of Sorghum bicolor L. used to prepare functional foods: a review on the efficacy against different chronic disorders International. *J Food Propert.* (2022) 25:1045–62. doi: 10.1080/10942912.2022.2071293

80. Rezaeinezhad A, Eslami P, Mirmiranpour H, Ghomi H. The effect of cold atmospheric plasma on diabetes-induced enzyme glycation, oxidative stress, and inflammation; in vitro and in vivo. *Sci Rep.* (2019) 9:1–11. doi: 10.1038/s41598-019-56459-y

81. Freund MA, Chen B, Decker EA. The inhibition of advanced glycation end products by carnosine and other natural dipeptides to reduce diabetic and age-related complications. *Comp Rev Food Sci Food Safety.* (2018) 17:1367–78. doi: 10.1111/1541-4337.12376

82. Podkowińska A, Formanowicz D. Chronic kidney disease as oxidative stress-and inflammatory-mediated cardiovascular disease. *Antioxidants.* (2020) 9:752. doi: 10.3390/antiox9080752

83. Houjeghani S, Kheirouri S, Faraji E, Jafarabadi MA. L-Carnosine supplementation attenuated fasting glucose, triglycerides, advanced glycation end products, and tumor necrosis factor–α levels in patients with type 2 diabetes: a double-blind placebo-controlled randomized clinical trial. *Nutr Res.* (2018) 49:96–106. doi: 10.1016/j.nutres.2017.11.003

84. Yan S-L, Wang Z-H, Mong M-C, Yang Y-C, Yin M-C. Combination of carnosine and asiatic acid provided greater anti-inflammatory protection for HUVE cells and diabetic mice than individual treatments of carnosine or asiatic acid alone. *Food Chem Toxicol.* (2019) 126:192–8. doi: 10.1016/j.fct.2019.02.027

85. Saryono, Proverawati A. The potency of black garlic as anti-atherosclerotic: Mechanisms of action and the prospectively. In: *AIP Conference Proceedings*. AIP Publishing LLC (2019).

86. Song H, Cui J, Mossine VV, Greenlief CM, Fritsche K, Sun GY, et al. Bioactive components from garlic on brain resiliency against neuroinflammation and neurodegeneration. *Exp Ther Med.* (2020) 19:1554–9. doi: 10.3892/etm.2019.8389

87. Khatana C, Saini NK, Chakrabarti S, Saini V, Sharma A, Saini RV, et al. (2020). Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. *Oxid Med Cell Longev.* (2020). doi: 10.1155/2020/5245308

88. Malaguarnera M. Acetyl-L-carnitine in hepatic encephalopathy. *Metab Brain Dis.* (2013) 28:193–9. doi: 10.1007/s11011-013-9376-4

89. Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis.* (2013) 4:e532–e532. doi: 10.1038/cddis.2013.60

90. Ali A, Mughal H, Ahmad N, Babar Q, Saeed A, Khalid W, et al. Novel therapeutic drug strategies to tackle immune-oncological challenges faced by cancer patients during COVID-19. *Expert Rev Anticancer Ther.* (2021) 21:1371–83. doi: 10.1080/14737140.2021.1991317

91. Shahidi F, Ramakrishnan VV, Oh WY. Bioavailability and metabolism of food bioactives and their health effects: a review. *J Food Bioact.* (2019) 8. doi: 10.31665/JFB.2019.8204

92. Yasin HK, Taylor AH, Ayakannu T. A narrative review of the role of diet and lifestyle factors in the development and prevention of endometrial cancer. *Cancers.* (2021) 13:2149. doi: 10.3390/cancers13092149

93. Abdel-Wahab AF, Mahmoud W, Al-Harizy RM. Targeting glucose metabolism to suppress cancer progression: prospective of anti-glycolytic cancer therapy. *Pharmacol Res.* (2019) 150:104511. doi: 10.1016/j.phrs.2019.104511

94. Panahi Y, Hosseini MS, Khalili N, Naimi E, Majeed M, Sahebkar A. Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: a randomized controlled trial and an updated meta-analysis. *Clin Nutr.* (2015) 34:1101–8. doi: 10.1016/j.clnu.2014.12.019

95. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front Physiol.* (2020) 11:694. doi: 10.3389/fphys.2020.00694

96. Damiano S, Muscariello E, La Rosa G, Di Maro M, Mondola P, Santillo M. Dual role of reactive oxygen species in muscle function: can antioxidant dietary supplements counteract age-related sarcopenia? *Int J Mol Sci.* (2019) 20:3815. doi: 10.3390/ijms20153815

97. Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant defence systems and oxidative stress in poultry biology: an update. *Antioxidants.* (2019) 8:235. doi: 10.3390/antiox8070235

98. Bigornia SJ, Mott MM, Hess DT, Apovian CM, Mcdonnell ME, Duess MA, et al. Long-term successful weight loss improves vascular endothelial function in severely obese individuals. *Obesity.* (2010) 18:754–9. doi: 10.1038/oby.2009.482

99. López-Domènech S, Martínez-Herrera M, Abad-Jiménez Z, Morillas C, Escribano-López I, Díaz-Morales N, et al. Dietary weight loss intervention improves subclinical atherosclerosis and oxidative stress markers in leukocytes of obese humans. *Int J Obes.* (2019) 43:2200–9. doi: 10.1038/s41366-018-0309-5

100. Van Baak MA, Mariman E. Mechanisms of weight regain after weight loss—the role of adipose tissue. *Nat Rev Endocrinol.* (2019) 15:274–87. doi: 10.1038/s41574-018-0148-4

101. Konstantinidi M, Koutelidakis AE. Functional foods and bioactive compounds: a review of its possible role on weight management and obesity's metabolic consequences. *Medicines.* (2019) 6:94. doi: 10.3390/medicines6030094

102. Akhlaghi M. Dietary Approaches to Stop Hypertension (DASH): Potential mechanisms of action against risk factors of the metabolic syndrome. *Nutr Res Rev.* (2020) 33:1–18. doi: 10.1017/S0954422419000155

103. Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-Mccomb J, et al. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. *J Nutr Biochem.* (2018) 58:1–16. doi: 10.1016/j.jnutbio.2018.02.012

104. Millman JF, Okamoto S, Teruya T, Uema T, Ikematsu S, Shimabukuro M, et al. Extra-virgin olive oil and the gut-brain axis: influence on gut microbiota, mucosal immunity, and cardiometabolic and cognitive health. *Nutr Rev.* (2021) 79:1362–74. doi: 10.1093/nutrit/nuaa148

105. Aoun A, Darwish F, Hamod N. The influence of the gut microbiome on obesity in adults and the role of probiotics, prebiotics, and synbiotics for weight loss. *Prev Nutr Food Sci.* (2020) 25:113. doi: 10.3746/pnf.2020.25.2.113

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A cross-sectional study on the effect of dietary zinc intake on the relationship between serum vitamin D_3 and HOMA-IR

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Introduction: Serum vitamin D_3 concentration is associated with the risk of insulin resistance. Zinc has also been reported to be associated with a lower risk of insulin resistance. In addition, zinc is an essential cofactor in the activation of vitamin D_3. However, the effect of dietary zinc intake on the relationship between vitamin D_3 and insulin resistance risk has not been fully studied. Therefore, we designed this cross-sectional study to assess the impact of changes in zinc intake on the relationship between vitamin D_3 and insulin resistance risk.

Study design and methods: This study analyzed data from the national Health and Nutrition Examination Survey (NHANES) from 2005 to 2018, involving 9,545 participants. Participants were stratified by zinc intake category (low zinc intake<9.58 mg/ day; High zinc intake: ≥9.58 mg/ day).

Results: In this cross-sectional study, serum vitamin D_3 levels were independently associated with the risk of insulin resistance in both the low and high Zinc intakes (β: −0.26, 95%CI: −0.56~0.04 vs. β: −0.56, 95%CI: −1.01~-0.11). In addition, this association was influenced by different dietary zinc intakes (interaction $P < 0.05$).

Conclusions: Our results suggest that zinc intake may influence the association between serum vitamin D_3 and the risk of insulin resistance. Further randomized controlled trials are needed to provide more evidence of this finding.

KEYWORDS

vitamin D3, zinc, insulin resistance, a cross-sectional study, interaction

Introduction

Insulin resistance is a part of abnormal cardiovascular metabolism, often referred to as "insulin resistance syndrome" or "metabolic syndrome," which may also accelerate the development of atherosclerosis, hypertension or polycystic ovary syndrome (1). Especially, Insulin resistance has been confirmed to be closely related to the onset of type 2 diabetes (T2D) (2). The increase in the prevalence of T2D has become a serious public health problem, resulting in an increase in related morbidity and mortality in addition to a huge economic burden (3). Therefore, it is necessary to identify the nutrients associated with insulin resistance to prevent insulin resistance.

Vitamin D deficiency is an epidemic (4) and has been linked to asthma, diabetes, cancer and neuropsychiatric disorders (5). In recent years, there have been numerous studies on the relationship between vitamin D and insulin resistance (6–9). However, the relationship between vitamin D_3 and insulin resistance remains controversial. The study of Mahendra Bhauraoji Gandhe et al. showed a significant negative correlation between vitamin D status and insulin level, suggesting that vitamin D supplementation may increase insulin sensitivity (10). However, the study of Zixin Xu et al. showed that such correlation varies between individuals and races (11). In addition, oral glucose tolerance tests conducted by Dilek Erdonmez et al. in some high school students showed no correlation between insulin measurements and vitamin D deficiency (12). These differences in findings may be due to potential confounding factors that have not been fully considered, such as dietary zinc intake.

Zinc has been reported to be associated with insulin resistance risk. Higher serum zinc concentrations are associated with increased insulin sensitivity (13). Zinc has specific functions in the biochemistry of insulin and glucagon in pancreatic β- and α- cells (14). In addition, the expression of the gene SLC30A10 encoding the zinc transporter ZnT10 was regulated by vitamin D_3 (15). It is concluded that zinc may be associated with the activation of vitamin D. However, the current study has not fully explored the effect of dietary zinc intake on the relationship between vitamin D_3 and insulin resistance risk. Therefore, in this cross-sectional study, we hypothesized that zinc and vitamin D_3 interact with insulin resistance. We aimed to investigate the effect of zinc intake on the association between vitamin D_3 and insulin resistance.

Methods

Data sources and study population

This is a cross-sectional study. We used data from the National Health and Nutrition Examination Survey (NHANES) continuously from 2005 to 2018. Participants included in the analysis were aged 20 years or older and had completed interviews and examinations in the mobile examination center (MEC). Participants with unknown serum vitamin D, insulin resistance, and covariates were excluded. NHANES (16) is a national health-related survey designed to assess the health and nutritional status of non-hospitalized US citizens. Survey participants selected representative samples of multi-stage stratified probability indicators (17). Extensive household interviews were conducted to gather demographic and health history information. A physical examination was performed and blood samples were collected in the MEC. The serum samples were analyzed in the United States by the Laboratory Science Division of the National Center for Environmental Health and the Centers for Disease Control and Prevention.

The study was approved by the Ethics Review Board of the National Center for Health Statistics Research. Before starting the study, the protocol was approved by the ethics board of the national review board CPP Sud-Méditerranée IV. Our research is based on public data from the NHANES, all details are from the official website (https://www.cdc.gov/nchs/nhanes/index.htm).

Zinc intake

Data on zinc dietary intake during the previous 24 h were collected through MEC's dietary review interview. Daily zinc intakes were divided into high and low intakes based on a median (9.58 mg/d). In large-scale surveys, 24-h recall (18) is the most commonly used dietary intake survey method. The decision to continue using the method at NHANES over the years was based on a consensus reached by the expert group at regular seminars to evaluate NHANES 'data collection methods (19).

Definition of insulin resistance

HOMA-IR is used to evaluate individual insulin resistance levels. The index was calculated as follows: fasting glucose level (FPG, mmol/L) × fasting insulin level (FINS, μU/mL) /22.5. Where, coefficient 22.5 is the correction factor, which refers to the blood glucose level of 4.5 mmol/L corresponding to 5 μU/mL plasma insulin in normal individuals. HOMA-IR reflects the interaction between glucose and insulin in different organs. The HOMA-IR index of normal individuals is 1. With the increase of insulin resistance level, the HOMA-IR index will be higher than 1.

Covariates

This article takes into consideration the age, sex, race/ethnicity, marital status, PIR, BMI, high-density lipoprotein cholesterol (HDL-c), education level, smoking

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status, physical condition, activity, alcohol, level of serum vitamin D_3, magnesium dietary intake, dietary zinc intake, dietary calcium intake, serum vitamin A level, total cholesterol, as A potential confounding factors. Race and ethnicity are divided into Mexican-Americans, non-Hispanic blacks, non-Hispanic whites, other Hispanics, and other races, including multiracial. Marital status is divided into married and unmarried, and married includes cohabitation, separation, divorce and widowhood. Smoking status is classified as current smokers, former smokers and never smokers. Participants who reported not smoking 100 cigarettes in their lifetime were considered never-smokers. Participants who smoked more than 100 cigarettes in their lifetime but did not currently smoke were considered former smokers. Reports have smoked more than 100 cigarettes in their lifetime and are now considered current smokers sometimes even daily. We define household income using the poverty income ratio (PIR), which is calculated from a specific threshold for household size. BMI is an internationally used measure of obesity and health, calculated from weight and height. Measure your weight in pounds on a digital scale and convert it to kilograms. Height is measured with an electronic tacheometer, accurate to millimeters. Education level includes below high school, high school graduation and university degree or above. Physical is classified as no or unknown, moderate or vigorous, according to whether moderate exercise caused a slight increase in respiration or heart rate, and vigorous exercise, fitness or recreational activities caused a significant increase in respiration or heart rate during the week. Work activity was classified according to three levels of activity intensity, non-work activity, moderate work activity and vigorous work activity. The drinking status of the drinkers and non-drinkers. Categorize drinkers as having more than 12 alcoholic drinks per year. Dietary recall interviews were conducted prior to MEC interviews to collect dietary information for the previous 24 h, including total dietary energy, vitamin D_3, magnesium, zinc, and calcium. Vitamin D_3 is the concentration in the serum.

Statistical analysis

All analyses were performed using statistical software package R (http://www.R-project.org, R Foundation). The relationship between serum vitamin D_3 concentration and insulin resistance was compared between low and high zinc intakes. We used the sample weight provided by NHANES. A hierarchical weighted multiple logistic regression model was used to subgroup zinc intake. Calculate the beta value and 95% confidence interval. The likelihood ratio test was used to examine the interactions between subgroups. A linear trend test was performed by entering the median value of each serum Vitamin D_3 as a continuous variable in the model. P-values <0 are considered statistically significant. The continuous variables are analyzed by t-test (Normal distribution) and Kruskal–Wallis (skewness distribution) tests.

Result

Baseline characteristics of the study population

Seven NHANES cycles were used in this study, namely 2005–2006, 2007–2008, 2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018. As shown in the flow chart (Figure 1), 70,190 potential participants were identified; among them, 39,749 adults ($\geq$20 years old) were included, excluding minor participants younger than 20 years old ($n = 30{,}441$); Participants with absent serum 25-hydroxyvitamin D concentration data and absent insulin resistance were excluded ($n = 27{,}165$). After excluding participants with missing covariant data, the remaining 9,545 participants were included in our analysis. A flowchart for exclusion criteria is shown in Figure 1. Table 1 shows the baseline characteristics of a study population divided into two groups based on dietary zinc intake. Individuals with high zinc intakes ($\geq$9.58 mg/day) were more likely to be male, Caucasian, younger, stronger, more physically active, more highly educated, and mostly with higher incomes than those with low zinc intakes (<9.58 mg/day). Individuals with high zinc intake drank less alcohol and had lower HDL levels. In terms of dietary factors, dietary calcium, magnesium, vitamin D_3 and vitamin A intakes were higher among participants with higher zinc intakes. There were no significant differences in marital status, BMI, smoking status and HDL levels between the high and low zinc intake groups.

Zinc intake affects the association between vitamin D_3 and insulin resistance

After adjusted for age, gender, race/ethnicity, BMI, education level, physical activity, smoking status, alcohol, PIR, marital status, HDL, total cholesterol, work activity, dietary magnesium intake, dietary calcium intake, dietary zinc intake, serum vitamin D_3 levels, dietary vitamin A intake, and magnesium had an interaction with the association between vitamin D_3 and insulin resistance (Table 2). Vitamin D_3 was used as a categorical variable. The two groups were divided into two groups: low level group ($\leq$58.3 nmol/L) and high level group (>58.3 nmol/L). In the high zinc intake group, the mean β value of insulin resistance of participants with serum vitamin $D_3 > 58.3$ nmol/L was −0.56 (95%CIS: −1.01–0.11, $P = 0.014$),

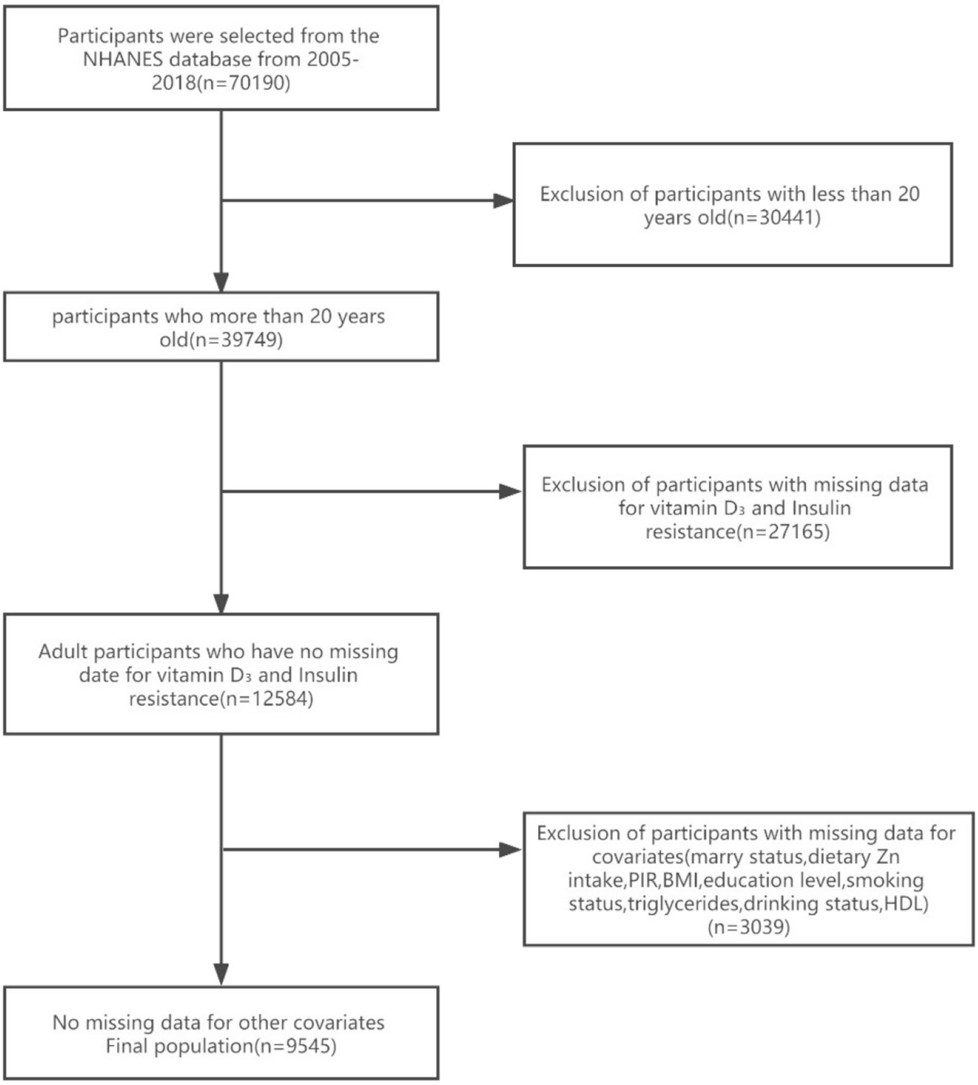

FIGURE 1
The flow chart of the study.

suggesting a correlation between dietary zinc intake and vitamin D_3 and insulin resistance (likelihood ratio test of interaction $P < 0.05$, $P < 0.05$). However, there was no significant difference in the low zinc intake group ($P = 0.092$). In addition, serum vitamin D_3 levels were further divided into three groups: low level group ($\leq$47.3 nmol/L), moderate level (47.3–69.8 nmol /L) and high level ($\leq$69.8 nmol/L). Among participants with high zinc intake, there was an independent association between serum vitamin D_3 levels and HOMA-IR index, and whether this association was affected by different zinc intake. Insulin resistance decreased significantly with increased vitamin D_3 concentration in the high-zinc group ($\beta = -1.04$, 95%CI: $-1.61 \sim -0.46$, $P < 0.001$) but not in the low-zinc group ($\beta = -0.27$, 95%CI: $-0.64 \sim 0.1$, $P = 0.154$).

Discussion

In a sample of adults over 20 years of age from the National Health and Nutrition Examination Survey (NHANES), our results suggest that serum vitamin D_3 levels were significantly higher in non-insulin-resistant participants with high zinc intake than in insulin-resistant participants. Serum vitamin D_3 concentration was inversely associated with insulin resistance risk in the high zinc intake group, but not in the low zinc intake group. We suspect that the correlation between serum vitamin D_3 concentration and the risk of insulin resistance is only apparent when zinc concentration reaches a certain threshold. In addition, a relationship between dietary zinc intake and vitamin D in the treatment of diabetes was also found (20), suggesting

TABLE 1 Baseline characteristics of participants.

Variables	Dietary zinc intake (mg/d) Total ($n = 9{,}545$)	Zinc ≤ 9.58 (mg/d) ($n = 4{,}769$)	Zinc > 9.58 (mg/d) ($n = 4{,}776$)	*p*
Age, median (IQR)	50.0 (34.0, 64.0)	52.0 (36.0, 66.0)	47.0 (34.0, 62.0)	<0.001
Gender, *n* (%)				<0.001
Female	4,852 (50.8)	2,957 (62)	1,895 (39.7)	
Male	4,693 (49.2)	1,812 (38)	2,881 (60.3)	
Race, *n* (%)				<0.001
Mexican American	1,415 (14.8)	638 (13.4)	777 (16.3)	
Non-Hispanic black	1,821 (19.1)	1,058 (22.2)	763 (16)	
Non-Hispanic white	4,405 (46.1)	2,050 (43)	2,355 (49.3)	
Other Hispanic	993 (10.4)	548 (11.5)	445 (9.3)	
Other race	911 (9.5)	475 (10)	436 (9.1)	
Marital status, *n* (%)				0.755
No	1,724 (18.1)	855 (17.9)	869 (18.2)	
Yes	7,821 (81.9)	3,914 (82.1)	3,907 (81.8)	
PIR, Mean ± SD	2.5 ± 1.6	2.4 ± 1.6	2.6 ± 1.6	<0.001
BMI, *n* (%)				0.158
<25	2,791 (29.2)	1,412 (29.6)	1,379 (28.9)	
25–29.9	3,221 (33.7)	1,565 (32.8)	1,656 (34.7)	
>30	3,533 (37.0)	1,792 (37.6)	1,741 (36.5)	
HDL, Mean ± SD	54.1 ± 16.0	55.4 ± 16.6	52.8 ± 15.2	<0.001
Education level, *n* (%)				<0.001
Less than high school	2,267 (23.8)	1,249 (26.2)	1,018 (21.3)	
High school graduation	2,161 (22.6)	1,103 (23.1)	1,058 (22.2)	
College or above	5,117 (53.6)	2,417 (50.7)	2,700 (56.5)	
Smoking status, *n* (%)				0.004
Never	5,244 (54.9)	2,640 (55.4)	2,604 (54.5)	
Former	2,376 (24.9)	1,123 (23.5)	1,253 (26.2)	
Now	1,925 (20.2)	1,006 (21.1)	919 (19.2)	
Physical activity, *n* (%)				< 0.001
No/Unknown	4,923 (51.6)	2,616 (54.9)	2,307 (48.3)	
Moderate	2,573 (27.0)	1,311 (27.5)	1,262 (26.4)	
Vigorous	2,049 (21.5)	842 (17.7)	1,207 (25.3)	
Work activity, *n* (%)				<0.001
Non-work activity	5,556 (58.2)	2,935 (61.5)	2,621 (54.9)	
Moderate work activity	2,154 (22.6)	1,058 (22.2)	1,096 (22.9)	
Vigorous work activity	1,835 (19.2)	776 (16.3)	1,059 (22.2)	
Alcohol, *n* (%)				<0.001
Yes	2,619 (27.4)	1,504 (31.5)	1,115 (23.3)	
No	6,926 (72.6)	3,265 (68.5)	3,661 (76.7)	
Serum Vitamin D_3, Mean ± SD	60.8 ± 27.0	59.4 ± 28.4	62.2 ± 25.4	<0.001
Magnesium intake, Mean ± SD	292.5 ± 147.3	218.0 ± 91.8	366.8 ± 154.6	<0.001
Zinc intake, Mean ± SD	11.1 ± 7.0	6.4 ± 2.0	15.8 ± 7.0	<0.001
Calcium intake, Median (IQR)	803.0 (519.0, 1163.0)	600.0 (398.0, 845.0)	1066.0 (750.0, 1469.0)	<0.001
Vitamin A intake, Median (IQR)	459.0 (256.0, 764.0)	333.0 (176.0, 556.0)	621.5 (380.0, 939.2)	<0.001
Total chol, Mean ± SD	191.8 ± 40.7	192.4 ± 41.3	191.2 ± 40.2	0.136

Data presented are ORs and 95% CIs. BMI, Body Mass Index; PIR, the ratio of family income to poverty; Total chol, total cholesterol.

TABLE 2 Interactive effect of vitamin D_3 and dietary zinc intake on insulin resistance (all participants).

Variable	Dietary intake zinc ≤ 9.58(mg/d) (*n* = 4,769)		Dietary intake zinc > 9.58 (mg/d) (*n* = 4,776)		*P* for interaction
Vitamin D_3 (nmol/l)	β (95CI%)	*P*-value	β (95CI%)	*P*-value	
Subgroups-1					0.034
≤58.3	0 (reference)		0 (reference)		
>58.3	−0.26 (−0.56∼0.04)	0.092	−0.56 (−1.01∼-0.11)	0.014	
Subgroups-2					0.001
≤47.3	0 (reference)		0 (reference)		
47.3–69.8	−0.21 (−0.56∼0.13)	0.225	−1.02 (−1.55∼-0.49)	<0.001	
>69.8	−0.27 (−0.64∼0.1)	0.154	−1.04 (−1.61∼-0.46)	<0.001	
Trend test	−0.14 (−0.32∼0.05)	0.147	−0.5 (−0.79∼-0.21)	0.001	

Adjusted for age, gender, race/ethnicity, BMI, education level, physical activity, smoking status, alcohol, PIR, marital status, HDL, total cholesterol, work activity, dietary magnesium intake, dietary calcium intake, dietary zinc intake, serum vitamin D_3 levels, dietary vitamin A intake.

that vitamin D adequacy and high zinc intake are greater than the sum of individual effects.

The main function of vitamin D_3 is to regulate bone metabolism and calcium phosphate homeostasis. In addition, vitamin D_3 may play an important role in maintaining pancreatic cell function, the study reports. It works by activating vitamin D receptors (VDR) and regulating insulin secretion through calcium channels located in pancreatic cells (9). Vitamin D_3 plays a role in the prevention of insulin resistance by improving insulin secretion, glucose metabolism, glucose tolerance, insulin sensitivity and inhibiting systemic inflammation. Interaction of 1,25 (OH)2 vitamin D with nVDR leads to transcription of insulin, cell structure, and growth genes. Two prospective cohort studies support our conclusions, showing that higher vitamin D concentrations are inversely associated with the risk of insulin resistance. Another study showed that vitamin D can indirectly stimulate insulin secretion and reduce the risk of insulin resistance by normalizing extracellular calcium by changing the calcium flow of cell membranes (21). A study by Nagashima et al. showed that 1, 25-dihydroxyl metabolites metabolized by liver and kidney hydroxylase could prevent quitiapine-induced insulin resistance *in vitro* through the PI3K signaling pathway (8). In a large Canadian cohort of non-diabetic adults, vitamin D status was found to be inversely associated with insulin responsiveness. Insulin response was associated with 25(OH) vitamin D levels in patients with baseline 25(OH) vitamin D levels ranging from 40 to 90 nmol/L, after adjusting for BMI, waist circumference, body weight, age, and sex (22). In addition, plasma vitamin D levels were negatively associated with classic parameters of obesity such as body mass index, fat mass and waist circumference. Notably, serum 25(OH) vitamin D levels were significantly lower in obese people than in lean people. Overweight and obese people were 25 percent and 35 percent more likely to have vitamin D deficiency than lean people, respectively (6).

However, Burnett et al. showed no association between total vitamin D intake and type 2 diabetes after adjustment for multiple potential confounders (23). A study examining the association between vitamin D and diabetes in the Thai population showed that the association between vitamin D and HbA1c was observed only in certain age groups (35–74 years), and that vitamin D deficiency had a significant effect only on older subjects living in an urban environment (24). It can be speculated that the relationship between vitamin D and insulin resistance is greatly affected by environmental factors. Similarly, Sadyia et al. reported no significant change in HbA1c percentage in the UAE population after 6 months of vitamin D supplementation (25). This may be due to the large differences between our sample and those in the clinical trials or due to the fact that the samples in the clinical trials did not control the levels of other factors that may affect vitamin D_3 absorption, thus influencing the effect of vitamin D_3 on insulin resistance.

Zinc is an essential trace element and micronutrient in human body and plays an important role in various physiological processes. Human needs for zinc are second only to those for iron. Its deficiency was significantly associated with induced oxidative stress, inflammatory events, and vascular dysfunction. Epidemiological studies have shown that low serum zinc levels are negatively correlated with a variety of diseases, such as diabetes, coronary artery disease and Parkinson's disease (26–32). Since 1934, when zinc was shown to be a component of insulin crystals, a link between zinc and diabetes has been proposed. Zinc plays a key role in insulin secretion and signaling. One study suggests that changes in biochemical parameters of zinc observed in obese individuals contribute to the presentation of related diseases, such as insulin resistance (8). In addition, several studies have established that zinc plays a fundamental role in insulin synthesis, storage, and action by stimulating its receptor, which protects liver and pancreatic cells from free radicals. Furthermore, as a nutrient that plays an important role in insulin sensitivity, zinc plays an indispensable role in maintaining the stability of insulin Homer (33). According to Jansen et al., disturbance of zinc homeostasis seems to be associated not only with diabetes, but also with several other diseases, such as cirrhosis, tumor, intestinal disease, and impaired immune system function (34).

A recent animal trial exploring the relationship between zinc supplementation and hyperglycemia and associated metabolic abnormalities revealed that following zinc supplementation, diabetic rats had a significant increase in plasma albumin, decrease in plasma urea and creatinine levels, and significant changes in insulin sensitivity indices HOMA-IR, HOMA-B, and QUICKI. Thus, this experiment provides the first evidence indicating that zinc supplementation can partially ameliorate the severity of diabetic hyperglycemia and the associated metabolic abnormalities, hypoinsulinemia, insulin resistance, and morphological changes of the pancreas, thereby inferring that zinc supplementation may offer significant potential for clinical applications in managing diabetic hyperglycemia and associated metabolic complications (35) which is consistent with our findings. Another study also revealed that zinc oxide nanoparticles act as effective antidiabetic agents (36).

Vitamin D_3 can directly affect cellular zinc homeostasis by inducing zinc transporters. In a study of cells treated with vitamin D, there was a 15 fold increase in the SLC30A10 gene, which is responsible for the translation of the zinc transporter znt10 protein. Vitamin D_3 can significantly alter the expression of various transporter genes involved in multiple physiological processes, including drug metabolism and transport, in Caco-2 cells. The SLC30A10 gene and its encoded protein znt10 were increased in Caco-2 cells treated with vitamin D_3, which may indicate a molecular correlation between vitamin D_3 levels and zinc regulation (15).

Given the prevalence and widespread supplementation of vitamin D deficiency, it would be interesting to understand the physiological and pharmacological implications of vitamin D_3 induction of SLC30A10/ZnT10. In theory, the end result would be a decrease in zinc levels in the cytoplasm, with an increase in zinc levels in the stored organelles and extracellular fluid. It is tempting to speculate that vitamin D_3 stimulates the release of these metal ions into the circulation, reaching organs in need, and helps vitamin D_3 perform some physiological functions. For example, vitamin D_3, zinc and manganese have bone protective effects and can stimulate bone formation (37). Zinc is considered as a supplement for the treatment and prevention of osteoporosis (38). It is not known whether there is a synergistic effect between vitamin D_3, zinc and manganese, but some pathological symptoms may indicate an important link between these nutrients. Vitamin D and zinc levels are particularly reduced in inflammatory bowel disease (IBD), celiac disease, and food-protein induced gastrointestinal allergy (FPGIA) (39–41). Although malabsorption is a hallmark of these intestinal diseases, the regulatory role of these nutrients, in which an imbalance in one nutrient affects the circulating concentration of another, cannot be ruled out. Maternal intake of vitamin D and zinc, as well as vitamin E deficiency, is also associated with childhood asthma (42), and monitoring and supplementation of zinc and vitamin D is considered as a potential way to control wheezing in children (43). Our current data confirm previous reports and reveal that vitamin D_3 plays some role in zinc homeostasis. Our current data confirm previous reports and reveal that vitamin D_3 plays some role in zinc homeostasis.

The study has some limitations. First, as a cross-sectional observational study, the associations found in this study may not lead to direct causation and may be confused by some other unmeasured variables. However, a number of potential confounding factors, including some dietary factors, were adjusted for in the logistic regression model. Second, although we used a large sample, the study was limited to US residents. Therefore, when extrapolating to other groups, it is necessary to take this aspect into due consideration. Third, recall and self-report bias may occur because dietary data were obtained from self-reported 24-h dietary recall. The same participant may result in inaccurate results if the sample is re-sampled at different times.

Conclusion

To sum up, although there is evidence that vitamin D_3, zinc, and there was a link between insulin resistance, but the existing data are not sufficient to prove that a lack of vitamin D_3 in reducing insulin resistance and related role in the pathogenesis of the metabolic syndrome and the general reasons, may not be enough to prove that vitamin D_3 supplements to treat insulin resistance and metabolic syndrome. We believe that long-term, well-designed interventional clinical trials should be initiated to better understand the therapeutic potential of vitamin D_3 supplementation in insulin-resistant subjects with vitamin D_3 deficiency, focusing on dosage, treatment duration, side effects, and short- and long-term outcomes.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

Author contributions

Thanks to BH, Z-yL, and YC for their contribution to data processing and topic selection, at the same time, thanks to Y-xS and S-qY for their contribution in writing. I am also grateful to J-lG for his contribution to consulting relevant literature. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Lebovitz HE. Insulin resistance: definition and consequences. *Exp Clin Endocrinol Diabetes.* (2001) 109(Suppl. 2):S135–48. doi: 10.1055/s-2001-18576

2. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev.* (2018) 98:2133–223. doi: 10.1152/physrev.00063.2017

3. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *Int J Med Sci.* (2014) 11:1185–200. doi: 10.7150/ijms.10001

4. Dobnig H. A review of the health consequences of the vitamin D deficiency pandemic. *J Neurol Sci.* (2011) 311:15–8. doi: 10.1016/j.jns.2011.08.046

5. Holick MF. Vitamin D deficiency. *N Engl J Med.* (2007) 357:266–81. doi: 10.1056/NEJMra070553

6. Garbossa SG, Folli F. Vitamin D, sub-inflammation and insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. *Rev Endocr Metab Disord.* (2017) 18:243–58. doi: 10.1007/s11154-017-9423-2

7. Mirhosseini N, Vatanparast H, Mazidi M, Kimball SM. Vitamin D supplementation, glycemic control, and insulin resistance in prediabetics: a meta-analysis. *J Endocr Soc.* (2018) 2:687–709. doi: 10.1210/js.2017-00472

8. Nagashima T, Shirakawa H, Nakagawa T, Kaneko S. Prevention of antipsychotic-induced hyperglycaemia by vitamin D: a data mining prediction followed by experimental exploration of the molecular mechanism. *Sci Rep.* (2016) 6:26375. doi: 10.1038/srep26375

9. Wenclewska S, Szymczak-Pajor I, Drzewoski J, Bunk M, Sliwińska A. Vitamin D supplementation reduces both oxidative DNA damage and insulin resistance in the elderly with metabolic disorders. *Int J Mol Sci.* (2019) 20:2891. doi: 10.3390/ijms20122891

10. Gandhe MB, Jain K, Gandhe SM. Evaluation of 25(OH) vitamin D3 with reference to magnesium status and insulin resistance in T2DM. *J Clin Diagn Res.* (2013) 7:2438–41. doi: 10.7860/JCDR/2013/6578.3568

11. Xu Z, Gong R, Luo G, Wang M, Li D, Chen Y, et al. Association between vitamin D3 levels and insulin resistance: a large sample cross-sectional study. *Sci Rep.* (2022) 12:119. doi: 10.1038/s41598-021-04109-7

12. Erdönmez D, Hatun S, Çizmecioglu FM, Keser A. No relationship between vitamin D status and insulin resistance in a group of high school students. *J Clin Res Pediatr Endocrinol.* (2011) 3:198–201. doi: 10.4274/jcrpe.507

13. Vashum KP, McEvoy M, Milton AH, Islam MR, Hancock S, Attia J. Is serum zinc associated with pancreatic beta cell function and insulin sensitivity in pre-diabetic and normal individuals? Findings from the Hunter Community Study. *PLoS ONE.* (2014) 9:e83944. doi: 10.1371/journal.pone.0083944

14. Maret W. Zinc in pancreatic islet biology, insulin sensitivity, and diabetes. *Prev Nutr Food Sci.* (2017) 22:1–8. doi: 10.3746/pnf.2017.22.1.1

15. Claro da Silva T, Hiller C, Gai Z, Kullak-Ublick GA. Vitamin D3 transactivates the zinc and manganese transporter SLC30A10 via the Vitamin D receptor. *J Steroid Biochem Mol Biol.* (2016) 163:77–87. doi: 10.1016/j.jsbmb.2016.04.006

16. Fain JA. NHANES. *Diabetes Educ.* (2017) 43:151. doi: 10.1177/0145721717698651

17. Zipf G, Chiappa M, Porter KS, Ostchega Y, Lewis BG, Dostal J. National health and nutrition examination survey: plan and operations, 1999–2010. Vital and health statistics. *Ser 1 Prog Collect Proc.* (2013) 1–37.

18. Foster E, Lee C, Imamura F, Hollidge SE, Westgate KL, Venables MC, et al. Validity and reliability of an online self-report 24-h dietary recall method (Intake24): a doubly labelled water study and repeated-measures analysis. *J Nutr Sci.* (2019) 8:e29. doi: 10.1017/jns.2019.20

19. Ahluwalia N, Dwyer J, Terry A, Moshfegh A, Johnson C. Update on NHANES dietary data: focus on collection, release, analytical considerations, and uses to inform public policy. *Adv Nutr.* (2016) 7:121–34. doi: 10.3945/an.115.009258

20. Story MJ. Essential sufficiency of zinc, ω-3 polyunsaturated fatty acids, vitamin D and magnesium for prevention and treatment of COVID-19, diabetes, cardiovascular diseases, lung diseases and cancer. *Biochimie.* (2021) 187:94–109. doi: 10.1016/j.biochi.2021.05.013

21. Lips P, Eekhoff M, van Schoor N, Oosterwerff M, de Jongh R, Krul-Poel Y, et al. Vitamin D and type 2 diabetes. *J Steroid Biochem Mol Biol.* (2017) 173:280–5. doi: 10.1016/j.jsbmb.2016.11.021

22. Heaney RP, French CB, Nguyen S, Ferreira M, Baggerly LL, Brunel L, et al. A novel approach localizes the association of vitamin D status with insulin resistance to one region of the 25-hydroxyvitamin D continuum. *Adv Nutr.* (2013) 4:303–10. doi: 10.3945/an.113.003731

23. Burnett BP, Pillai L, Bitto A, Squadrito F, Levy RM. Evaluation of CYP450 inhibitory effects and steady-state pharmacokinetics of genistein in combination with cholecalciferol and citrated zinc bisglycinate in postmenopausal women. *Int J Womens Health.* (2011) 3:139–50. doi: 10.2147/IJWH.S19309

24. Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. The association between vitamin D status and type 2 diabetes in a Thai population, a cross-sectional study. *Clin Endocrinol.* (2012) 77:658–64. doi: 10.1111/j.1365-2265.2012.04422.x

25. Al-Sofiani ME, Jammah A, Racz M, Khawaja RA, Hasanato R, El-Fawal HA, et al. Effect of vitamin D supplementation on glucose control and inflammatory response in type II diabetes: a double blind, randomized clinical trial. *Int J Endocrinol Metab.* (2015) 13:e22604. doi: 10.5812/ijem.22604

26. Choi S, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. *Acta Pharmacol Sin.* (2018) 39:1120–32. doi: 10.1038/aps.2018.25

27. Du K, Liu MY, Zhong X, Wei MJ. Decreased circulating Zinc levels in Parkinson's disease: a meta-analysis study. *Sci Rep.* (2017) 7:3902. doi: 10.1038/s41598-017-04252-0

28. Frassinetti S, Bronzetti G, Caltavuturo L, Cini M, Croce CD. The role of zinc in life: a review. *J Environ Pathol Toxicol Oncol.* (2006) 25:597–610. doi: 10.1615/JEnvironPatholToxicolOncol.v25.i3.40

29. Gammoh NZ, Rink L. Zinc in infection and inflammation. *Nutrients.* (2017) 9:624. doi: 10.20944/preprints201705.0176.v1

30. Mendes Garrido Abregú F, Gobetto MN, Juriol LV, Caniffi C, Elesgaray R, Tomat AL, et al. Developmental programming of vascular dysfunction by prenatal and postnatal zinc deficiency in male and female rats. *J Nutr Biochem.* (2018) 56:89–98. doi: 10.1016/j.jnutbio.2018.01.013

31. Sahin O, Elcik D, Dogan A, Cetinkaya Z, Oguzhan A. The relation of serum trace elements and coronary atherosclerotic progression. *Trace Elem Electrol.* (2019) 36:210–4. doi: 10.5414/TEX01570

32. Samadi A, Isikhan SY, Tinkov AA, Lay I, Doşa MD, Skalny AV, et al. Zinc, copper, and oxysterol levels in patients with type 1 and type 2 diabetes mellitus. *Clin Nutr.* (2020) 39:1849–56. doi: 10.1016/j.clnu.2019.07.026

33. Morais JBS, Severo JS, Beserra JB, de Oiveira ARS, Cruz KJC, de Sousa Melo RS, et al. Association between cortisol, insulin resistance and zinc in obesity: a mini-review. *Biol Trace Element Res.* (2019) 191:323–30. doi: 10.1007/s12011-018-1629-y

34. Jansen J, Karges W, Rink L. Zinc and diabetes–clinical links and molecular mechanisms. The Journal of nutritional biochemistry (2009) 20:399–417. doi: 10.1016/j.jnutbio.2009.01.009

35. Barman S, Srinivasan K. Zinc supplementation alleviates hyperglycemia and associated metabolic abnormalities in streptozotocin-induced diabetic rats. *Can J Physiol Pharmacol.* (2016) 94:1356–65. doi: 10.1139/cjpp-2016-0084

36. Nazarizadeh A, Asri-Rezaie S. Comparative study of antidiabetic activity and oxidative stress induced by zinc oxide nanoparticles and zinc sulfate in diabetic rats. *AAPS PharmSciTech.* (2016) 17:834–43. doi: 10.1208/s12249-015-0405-y

37. Zofková I, Nemcikova P, Matucha P. Trace elements and bone health. *Clin Chem Labor Med.* (2013) 51:1555–61. doi: 10.1515/cclm-2012-0868

38. Yamaguchi M. Role of nutritional zinc in the prevention of osteoporosis. *Mol Cell Biochem.* (2010) 338:241–54. doi: 10.1007/s11010-009-0358-0

39. Meyer R, De Koker C, Dziubak R, Godwin H, Dominguez-Ortega G, Shah N. Dietary elimination of children with food protein induced gastrointestinal allergy - micronutrient adequacy with and without a hypoallergenic formula? *Clin Transl Allergy.* (2014) 4:31. doi: 10.1186/2045-7022-4-31

40. Oxentenko AS, Murray JA. Celiac disease: ten things that every gastroenterologist should know. *Clin Gastroenterol Hepatol.* (2015) 13:1396–404. doi: 10.1016/j.cgh.2014.07.024

41. Santucci NR, Alkhouri RH, Baker RD, Baker SS. Vitamin and zinc status pretreatment and posttreatment in patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* (2014) 59:455–7. doi: 10.1097/MPG.0000000000000477

42. Allan K, Devereux G. Diet and asthma: nutrition implications from prevention to treatment. *J Am Diet Assoc.* (2011) 111:258–68. doi: 10.1016/j.jada.2010.10.048

43. Uysalol M, Uysalol EP, Yilmaz Y, Parlakgul G, Ozden TA, Ertem HV, et al. Serum level of vitamin D and trace elements in children with recurrent wheezing: a cross-sectional study. *BMC Pediatr.* (2014) 14:270. doi: 10.1186/1471-2431-14-270

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Plant-based default nudges effectively increase the sustainability of catered meals on college campuses: Three randomized controlled trials

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Background: Literature suggests limiting consumption of animal products is key to reducing emissions and adverse planetary impacts. However, influencing dietary behavior to achieve planetary health targets remains a formidable problem.

Objective: We investigated the effect of changing the default meal option at catered events–from meat to plant-based–on participants' meal choices using three parallel-group, balanced, randomized controlled trials (RCT), and use these experimental results to project differences in plant-based default vs. meat default events on greenhouse gas emissions (GHGEs) (kg CO_{2-eq}), land use (m^2), nitrogen (g N), and phosphorus (g P) footprint.

Methods: Data collection was performed at three catered events ($n = 280$) across two college campuses. The selected experimental sites used convenience sampling. Events consisted of a graduate orientation, sorority dinner, and academic conference. Eligibility of individual participants included being 18 years or older and an invitation to RSVP for an enrolled event. Participants were randomly assigned to one of two groups: the control group received a RSVP form that presented a meat meal as the default catering option; whereas the intervention group received a form that presented a plant-based meal as the default. The primary outcome of interest in each group was the proportion of participants who selected plant-based meals. To explore environmental impacts, we modeled the footprints of four hypothetical meals. Using these meals and RCT results, the impact (GHGE, land use, nitrogen, phosphorus) of two hypothetical 100-person events was calculated and compared.

Results: In all, participants assigned to the plant-based default were 3.52 (95% CI: [2.44, 5.09]) times more likely to select plant-based meals than those assigned to the meat default. Using these results, a comparison of hypothetical events serving modeled meat-based and plant-based meals showed a reduction of up to 42.3% in GHGEs as well as similar reductions in land use (41.8%), nitrogen (38.9%), and phosphorus (42.7%).

Conclusion: Results demonstrated plant-based default menu options are effective, providing a low-effort, high-impact way to decrease consumption of animal products in catered events. These interventions can reduce planetary impact while maintaining participant choice.

KEYWORDS

meat consumption, sustainable diet, default nudge, planetary boundary, carbon footprint, environmental impact, choice architecture

Introduction

Human-driven activities in food production, distribution, storage, consumption, and disposal account for roughly 23% of global greenhouse gas emissions (GHGEs). A disproportionate contribution of food-related emissions occurs in animal agriculture, which results in more than half of food-related emissions despite representing far less than half of average daily caloric intake in most societies (Gerber et al., 2013; Allen and Hoff, 2019; IPCC, 2020). A growing body of literature suggests that current food consumption patterns are unsustainable and if not modified, will prevent humanity from staying within established targets for anthropogenic climate change (Springmann et al., 2018a; Clark et al., 2020). A host of changes are needed across the food system and among consumers in order to establish a resilient food system and help combat these negative planetary health impacts.

Demand-side changes, such as adopting a flexitarian or plant-based diet, defined as "..fruits, vegetables, whole grains, legumes, nuts, seeds, herbs, and spices and excludes all animal products" (Ostfeld, 2017), represent impactful strategies for mitigating GHGEs and other environmental footprints (Hallström et al., 2015; Springmann et al., 2018b) [We define plant based as Ostfeld, 2017 does: a diet that consists of "…fruits, vegetables, whole grains, legumes, nuts, seeds hers, and spices and excludes all animal products."; though it is sometimes also used in context with diets that include animal products such as the Mediterranean diet (Scoditti et al., 2022)]. However, to date, efforts to promote the adoption of more plant-heavy diets have yielded little success (Vizcaino et al., 2020). Even among those who identify as vegan or vegetarian-roughly 10% of the global population-many are unable to maintain these diets consistently (Herzog, 2014; Nezlek and Forestell, 2019). This is despite the fact that plant-based food choices, in particular meat-replacement products, have grown considerably in the marketplace (Godfray and Oxford Martin School, 2019; Tzivia et al., 2020). Therefore, more research is needed to find low-cost and effective strategies for changing dietary behavior.

Choice architecture

While individuals' dietary choices are a reflection of important factors such as economic, social, cultural, and infrastructural influences, there are relatively simple, low cost strategies that can influence consumers to make more sustainable decisions. Choice architecture tools, or "nudges," are a promising set of interventions that can change behaviors by influencing the social, physical, or psychological environment in which people make choices (Thaler and Sunstein, 2008). Nudging aims to influence people's behaviors by changing the way an individual choice is presented, without restrictions or consumer awareness of the influence (Vandenbroele et al., 2019). More specifically, a wide range of choice architecture tools have been implemented and shown to promote environmentally friendly and healthy behaviors across a variety of settings (Garnett et al., 2019; Rare the Behavioural Insights Team, 2019).

One particularly effective choice architecture tool is the default nudge (i.e., the preselected option on a survey or form). Defaults leverage the human tendency to choose the path of least resistance (Van Gestel et al., 2020) to effect behavioral change. The default option also implies that the preselected option is the recommended choice (Carroll et al., 2009; Jachimowicz et al., 2019), which further motivates individuals to stay with the default. Research has shown that defaults have effectively achieved desired outcomes for organ donation, retirement savings, and green energy consumption in multiple countries (Thaler and Benartzi, 2004; Abadie and Gay, 2006; Pichert and Katsikopoulos, 2008).

Sustainable defaults

The default nudge is part of a specific class of choice architecture tools that are especially effective in the domain of food behaviors (Campbell-Arvai et al., 2012; Mertens et al., 2022). Defaults have been shown to shift diets toward healthier and more sustainable food options, which suggests that they might be effective for reducing meat consumption (Vecchio and Cavallo, 2019; Parkin and Atwood, 2022). Despite this promising evidence, a recent literature review by Meier's et al. (2021) emphasized that research on default nudges specifically for reducing meat consumption is still limited. Furthermore, most existing default studies targeting dietary behavior have been conducted in Europe and the majority utilized defaults in menu design or by controlling portion sizes (Campbell-Arvai et al., 2012; Meier's et al., 2021; Perez-Cueto, 2021). Additionally, only one study has attempted to quantify the potential environmental impacts based on the results of their experiment (Kurz, 2018).

Large institutional events are places where reducing individual meat consumption through the default nudge could scale to have a significant impact. For example, previous experiments among participants ($n = 330$) at three higher education conferences in Denmark revealed that plant-based meal selections in online pre-conference RSVP requests increased by 81-percentage points when a vegetarian buffet was presented as the default choice (Hansen et al., 2021). However, research testing the effectiveness of the default nudge to increase plant-based meal consumption is limited. To address the limitations in existing literature, the current study was designed to evaluate the effect of plant-based default nudges by: (1) testing the efficacy of plant-based meal defaults at three events on college campuses in the U.S., (2) estimating the environmental impact of the experimental results, and (3) comparing the impacts to the per-meal per-capita planetary boundaries suggested by the EAT-Lancet Commission's Global Planetary Health Diet (Willett et al., 2019; Hansen et al., 2021). The novelty of this work stems from its setting in US higher education institutions and modeling the potential environmental footprint savings possible from utilizing the default nudge.

Methods

We conducted a two part study. First, we carried out three parallel, [1:1] balanced RCTs. Then we quantified the potential environmental impact reduction of these interventions using modeled meals and lifecycle assessment (LCA) data. The RCT portion of our study was an extension of the methodology of Hansen et al. (2021). Whereas Hansen tested the impact of a plant-based default on three buffets at academic conferences, we tested the default on two individual meals and one buffet at various campus-related events.

TABLE 1 Details for three catered events included in study.

Campus	Department or Organization	Event description	*n*
Harvard	N/A	Workshop on Behavioral Insights in Health (BIH)	91
UCLA	Civil and Environmental Engineering (CEE)	Graduate student orientation	108
UCLA	Panhellenic Sorority	DG Monday night dinner	81

Randomized trials

Eligible participants were adults over the age of 18 who attended one of our three events. Data was collected at three independent events held at the University of California at Los Angeles (UCLA) and Harvard University, two higher education institutions in the U.S. The two events at UCLA were held in 2021: a graduate orientation for the Civil and Environmental Engineering Department and a dinner at a UCLA-affiliated sorority. The event at Harvard was a workshop on Behavioral Insights in Health (BIH) in 2017 (Table 1). The events would have been planned and held regardless of study enrollment. No demographic information was collected about participants.

At each event, event operators (EOs) decided which participants to invite, how the RSVP survey would be distributed, and which catering options to choose. The default intervention was implemented through a question on the RSVP survey. EOs sent participants a link to a Google script that randomized them into two groups: a control group and an intervention group, using a randomization function. This function then rendered one of two RSVP surveys in Google forms. Simple randomization was conducted in real-time when participants clicked on the RSVP survey link. A Google script was programmed to use the Math.floor() and Math.random() functions to select and render one of two links in an array of web links containing the survey options (control and intervention). Randomization was balanced between groups without blocking or stratification.

Each survey was identical save for the question about meal preferences for the event. No questions were asked prior to the meal preference question and the question was required. The control group received a question stating that the default meal contained meat, and participants were required to opt out if they desired a plant-based meal. Alternatively, the experimental group received the same survey with a variation of the meal preference question. The experimental group received a plant-based default meat option and participants were required to opt out if they wanted a meal containing meat.

The interventions themselves employed similar sentence structure, although wording differed slightly across events.

Specific wording of the intervention question can be found in the Supplementary material in section I. The RSVP survey purposefully conveyed endorsement and endowment by clearly stating the default meal option (plant-based or meat). The survey also required participants to click a button to select an alternative option if they desired a different meal (plant-based or meat). The buffet event RSVP survey took this process a step further by indicating that the default meal would be in the form of a buffet (where participants presumably would be given more options) and provided a space where participants could request an individual meal with no guidance on what that meal might be. The Harvard-BIH EOs interpreted plant-based as vegetarian—a superset of plant-based foods—in their RSVP survey, whereas the other events used our definition of plant-based. Hypothetical meals were analyzed with both plant-based and vegetarian meals to account for this discrepancy.

The implementation of the plant-based default and the actual menu items at each event differed. However, the primary outcome of interest in each group was the proportion of participants who selected plant-based meals. That proportion was used to calculate the environmental impact of hypothetical menu scenarios. The serving method and actual foods distributed at each event were not necessary for the goals of this research.

Caveat that Harvard event operators interpreted plant based as vegetarian. Whereas the other events used our definition of plant based. Hypothetical meals were analyzed with both fully plant based and vegetarian to account for this discrepancy.

Statistical analysis

The primary outcome was the proportion of participants who selected a plant-based meal. This was assessed by counting meal selections in each group among participants who indicated that they planned to attend the event. To compare the control and intervention groups, we used R (R Core Team, 2022) to fit two models within each site (Stapleton, 2009). First, we fit a log-binomial model to obtain risk ratios, a ratio of the probability that a participant in the intervention group selected the plant-based meal option to the probability that a participant in the control group would do so. Risk ratios >1 indicate that the intervention effect was in the desired direction. Second, we fit a linear regression model with heteroskedasticity-consistent robust standard errors to obtain differences in probabilities of plant-based meal selection within each group (White, 1980; Kleiber and Zeileis, 2008; Zeileis et al., 2020). To aggregate the results of all three RCTs and address differences in context, we fit the same two models to the data from all sites combined. This model includes fixed effects of each site to account for clustering of participants in each event (Stapleton, 2009).

Environmental footprint

To estimate environmental impact, we created four iso-caloric model sandwiches: one beef, one chicken, one cheese, and one tofu and bean sandwich. The conversion factors used to compute these environmental impacts of each food ingredient were based on lifecycle assessment values listed in section 3 in the Supplementary material. The calculations for each ingredient were then aggregated in Excel to determine the total impacts of each meal. The four sandwiches used represented meals served at a typical "boxed lunch" event. These sandwiches were based on existing catering menus from a popular sandwich shop and consisted of a plant-based sandwich (with mushrooms and a black bean-soy patty), a vegetarian sandwich (a vegetable sandwich similar to the plant-based but with mozzarella cheese instead of the patty), a chicken club (chicken and bacon sandwich) and a standard roast beef sandwich. These "standardized" model sandwiches were chosen because each experimental event provided different menus, and in some cases, what was served and consumed at the event was not known by researchers involved. The recipes used for our standard sandwiches are included in the Supplementary material. Meals were standardized to include comparable total calories (around 650) and contained a minimum of 30 g of protein to simulate an average protein rich sandwich. The meals were then used to model the potential impacts of each event (Table 2).

TABLE 2 Single-meal footprint calculations, caloric levels, and protein.

	Environmental impacts (percentages)					
	GHG g CO2-eq	Land use m2	Nitrogen g N	Phosphorus g P	Calories kcal	Protein g
Plant-based(Bean) Sandwich	410 (90%)	1.44 (121%)	4.59 (56%)	0.99 (135%)	655	30.0
Vegetarian (Cheese) Sandwich	980 (215%)	1.62 (136%)	13.7 (167%)	3.19 (437%)	647	29.4
Chicken and Bacon Sandwich	1,040 (228%)	2.77 (233%)	18.8 (228%)	3.47 (475%)	651	38.8
Beef Sandwich	3,840 (840%)	12.4 (1,040%)	27 (329%)	9.88 (1,353%)	648	37.5

Percentage of per-capita planetary boundary threshold in parentheses.

We specified 8 hypothetical catered events, each with 100 participants. These 8 events represented all possible combinations of 2 meat meals (beef or chicken), 2 non-meat meals (vegetarian or plant-based), and 2 defaults (default meat or default non-meat). For example, the first hypothetical event offered, by default, a beef sandwich, but offered the option to opt into receiving a plant-based sandwich instead.

To compare the footprints of these hypothetical events, we estimated the number of meat and non-meat selections at each event based on the effect size estimates obtained from the log-binomial model of data aggregated across sites. We used the planetary boundary framework as a standard model (Table 3). The conversion factors used to calculate the environmental footprints of each food ingredient were based on LCA values listed in section III of the Supplementary material. We then extrapolated the GHGEs, land usage, nitrogen usage, and phosphorus footprints of each event for comparison.

Results

Randomized trials

In all events, the intervention substantially increased plant-based meal selections (Table 4; Figure 1). In the two non-buffet events, Harvard-BIH ($n = 91$) and UCLA-CEE ($n = 108$), participants had 2.75 (1.59, 4.79) and 4.04 (2.04, 7.99) times the likelihood of selecting a plant-based meal in the intervention group vs. in the control group. Furthermore, the event with RSVP wording signaling that the default was a buffet was the most effective, with a risk ratio of 4.18 of participants selecting a plant-based meal in the intervention group. No recorded harms or unintended effects were reported.

TABLE 3 Per capita per meal planetary boundary (PB) thresholds as specified by the EAT-Lancet commission (Willett et al., 2019).

PB	PB for food system	PB per capita per meal
Climate change	5 Gtons year^{-1}	457 g CO_{2-eq} person^{-1} meal^{-1}
Land use	13 million km^2	1.18 m^2 land person^{-1} meal^{-1}
Blue water use	2,500 km^3 year^{-1}	0.228 m^3 person^{-1} meal^{-1}
Nitrogen	90 Tg N year^{-1}	8.23 g person^{-1} meal^{-1}
Phosphorus	8 Tg P year^{-1}	0.73 g P person^{-1} meal^{-1}

Environmental footprint

We calculated the potential environmental impacts for the 8 hypothetical events (Table 5). Under each meat and plant-based meal combination, group 1 represents a hypothetical 100-person event based on the observed meal selection of all participants in the control group (meat default) aggregated across all three RCTs. Group 2 also represents a hypothetical 100-person event but is instead based on the observed meal selection of all participants in the intervention group (plant-based default) aggregated across all three RCTs. These projected impacts were then used to calculate the potential differences between group 1, the hypothetical default meat event, and group 2, the hypothetical default veg event (Table 6). The 100-person hypothetical event was used for ease of calculation for modeled projections and does not reflect the number of participants from the RCT experiments.

None of the hypothetical events fell within the proposed limits of the planetary boundaries defined by EAT Lancet. However, the hypothetical, plant-based default event serving plant-based and chicken sandwiches aligned the most closely with these boundaries for GHGEs (150%), land use (171%), nitrogen (131%) and phosphorus (283%) (Figures 2A–D).

The meat-default and plant-based default events serving plant-based and beef sandwiches displayed the largest difference in environmental impacts. Yet the impact of the plant-based default event in this category was still relatively high for GHGEs, land use, and phosphorus due to beef's large environmental impacts in comparison to other ingredients. We found that the default-plant-based and default-vegetarian

TABLE 4 Percentages, risk ratios, and risk differences of plant-based meal selection among control and intervention groups for three events.

Primary outcome: Plant-based meal selection	Default meat		Default veg		Risk ratio (95% CI)	Risk difference (percentage points) (95% CI)
	N	Percentage (no)	N	Percentage (no)		
Harvard-BIH† ($N = 91$)	45	24.4% (11)	46	67.4% (31)	2.75 (1.59, 4.79)	42.9 (24.2, 61.7)
UCLA-CEE† ($N = 108$)	56	14.3% (8)	52	57.7% (30)	4.04 (2.04, 7.99)	43.4 (27, 59.9)
UCLA-DG† ($N = 81$)	40	17.5% (7)	41	73.2% (30)	4.18 (2.08, 8.40)	55.7 (37.4, 73.9)
All sites† ($n = 280$)	141	18.4% (26)	139	65.5% (91)	3.52 (2.44, 5.09)	46.8 (28.8, 64.8)

† $p < 0.0001$.

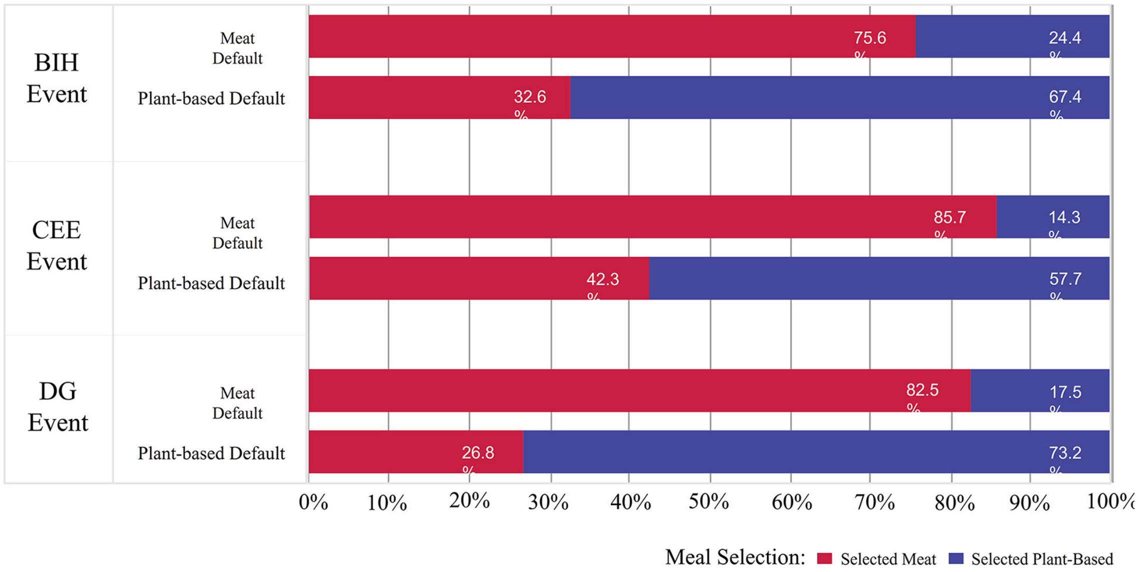

FIGURE 1
Percentages of plant-based and meat meal selection among control (meat default) and intervention groups (plant-based) for events.

TABLE 5 Estimated environmental impact in terms of GHG emissions, land use (LU), blue water (BW), nitrogen (N), and phosphorus (P) use for hypothetical 100-person events at a college campus.

	Environmental impacts (percentage of PB[†])			
	GHGEs $kg\ CO_{2-eq}$	LU m^2	N g	P g
Beef and Plant-based Sandwiches				
Group 1: Default beef, Opt-in plant-based[a]	328 (719%)	1,060 (902%)	2,340 (285%)	845 (1,160%)
Group 2: Default plant-based, Opt-in beef [b]	189 (414%)	619 (525%)	1,430 (174%)	484 (663%)
Chicken and Plant-based Sandwiches				
Group 1: Default chicken, Opt-in plant-based	94 (206%)	256 (217%)	1,650 (201%)	307 (421%)
Group 2: Default plant-based, Opt-in chicken	68.3 (150%)	202 (171%)	1,070 (131%)	206 (283%)
Beef and Vegetarian Sandwiches				
Group 1: Default beef, Opt-in vegetarian	338 (739%)	1,070 (904%)	2,490 (303%)	880 (1,210%)
Group 2: Default vegetarian, Opt-in beef	222 (485%)	629 (533%)	1,950 (237%)	60 (834%)
Chicken and Vegetarian Sandwiches				
Group 1: Default chicken, Opt-in vegetarian	103 (226%)	258 (219%)	1,800 (219%)	343 (469%)
Group 2: Default vegetarian, Opt-in chicken	101 (220%)	212 (179%)	1,590 (194%)	331 (454%)

[a]The footprints of group 1 are based on the aggregated selections of participants who received a meat default (16 selected plant based, 84 selected meat).
[b]The footprints of group 2 are based on the aggregated selections of participants who received a plant-based default (57 selected plant based, 43 selected meat).
[†] 100% representing the planetary boundary denoted by EAT-Lancet.

events had substantially lower carbon emissions, land-use, phosphorus, and nitrogen footprints than default-meat events. For example, in the event that serves beef sandwiches and plant-based sandwiches, implementing a plant-based default is projected to reduce GHGEs by 42.3% (139 grams) of CO2-eq. We also found a projected savings of 41.8% (445 m^2) in land-use, 38.9% (912 g) for nitrogen, and 42.7% (361 g) for phosphorus. Even when the meat sandwiches are chicken rather than beef, implementing a plant-based default was still projected to substantially improve on all environmental metrics. Compared to default-meat events, default-vegetarian events also improved on all five environmental metrics, though the improvements were somewhat smaller than for default-plant-based events.

TABLE 6 Projected improvement in hypothetical planetary boundary impacts when using a vegetarian or plant-based default meal vs. a meat default meal.

	Environmental impacts			
	GHGEs Kg CO_{2-eq}	LU m^2	N g	P g
Beef and Plant-based Sandwiches				
Projected difference[a]	139	445	912	361
Projected percent change	42.3%	41.8%	38.9%	42.7%
Chicken and Plant-based Sandwiches				
Projected difference[a]	25.6	54	577	101
Projected percent change	27.3%	21.1%	34.9%	32.8%
Beef and Vegetarian Sandwiches				
Projected difference[a]	116	438	540	272
Projected percent change	34.4%	41.1%	21.7%	30.9%
Chicken and Vegetarian Sandwiches				
Group 2 and Group 1 difference[a]	2.51	46.7	205	11.4
Percent change	2.43%	18.1%	11.4%	3.32%

[a]Obtained by subtracting projected impacts for the plant-based- or vegetarian-default from impacts for the meat-default in Table 2.

Discussion

Randomized controlled trials

The goal of this study was to test the effect of a plant-based default nudge on participant meal selection at catered events and quantify potential environmental impacts of the intervention. In the RCTs, we found significant, large effect sizes across all three experiments. At the Harvard-BIH, UCLA-CEE, and UCLA-DG events, the default nudge increased plant-based meal selection by 43, 43 and 56 percentage points, respectively. Across all sites, plant-based meal selection increased by 47 percentage points.

The effect sizes we observed were substantially larger than those of existing interventions targeting eating behaviors. Mertens et al.'s meta-analysis of choice architecture interventions reported an average standardized mean difference (SMD) of 0.72 for interventions in the domain of food (2022). This is approximately equivalent to a risk ratio of 1.92 (Chinn, 2000; VanderWeele, 2020). In studies using decision structure—the class of choice architecture tools to which the default nudge belongs—to affect food behaviors, the review reported a SMD of 0.86 or approximately a risk ratio of 2.17. However, our pooled risk ratio for all three studies was 3.52. Our risk ratio is relatively high among choice architecture studies aiming to influence food behavior but this may also be attributable to differences in the variables measured.

The effectiveness of default nudges can be attributed to a variety of factors: cognitive capacity due to aspects such as time pressures and selection effort; biases such as the endowment effect and the omission bias; as well as the perception that a default is an "implicit recommendation" of one choice over another (Michalek et al., 2015; Jachimowicz et al., 2019). Interventions leveraging these factors in varying ways could elicit different results.

For example, in Meier's et al. (2021) systematic literature review on plant-based defaults, virtually all studies found that the default intervention decreased meat consumption. However, the design, implementation, and results of these interventions differ greatly. One study found that changing a plant-based menu item to the dish of the day increased plant-based meal selections by 76 percentage points (Perez-Cueto, 2021). Garvert and Kurz's 2019 study found that rearranging the meal options led to a decreased probability of meat selection from 45.7 to 21.4%. Another study reordering meals presented at a cafeteria counter increased vegetarian meals selected, but only if the vegetarian and meat meals were placed far apart (Garnett et al., 2019). Parkin and Atwood (2022) found that for menus to effectively encourage diners to choose vegetarian options over meat, menus needed to be at least 75% vegetarian options. A more recent publication by Nykänen et al. (2022) also found that two experimental nudges intended to reduce red meat consumption (a "dish of the day" nudge approach, and "sequence alteration" approach) had no effect on the choices made for the main dish, nor the proportion of meat in the overall meal weight. Of the experiments designed to reduce meat consumption, most opted to do so by altering portion sizes, reordering items listed on a menu, or altering the descriptions of foods in restaurants and cafeterias (Meier's et al., 2021; Perez-Cueto, 2021; Nykänen et al., 2022). This demonstrates the importance of further studies to explore settings in which the default is effective and those where it is not. Additionally, most existing literature is difficult to compare to our study because they measured the amount of meat consumed *by weight* as opposed to measuring selection of a meal *containing* meat.

Our study was closely modeled after a plant-based default intervention conducted over the three academic conferences in Denmark (Hansen et al., 2021). Hansen et al. (2021) plant-based default was carried out through an RSVP for three academic conferences in Denmark. Hansen's team found 85, 80, and 77 percentage point differences in plant-based meal consumption across the three events. The results of each event were larger than our overall 47 percentage point difference. A number of factors could account for this difference. Hansen's default nudge implied increased variety of food items through the buffet wording in the RSVP (with a reported total of 330 food choices between the three events). In addition, while Hansen's events were academic conferences held in Denmark, our events were highly-varied university affiliated events held in the U.S. As a result, the social contexts in which these events occurred are different, with Denmark being consistently ranked as one of the top four most sustainable countries in the world and the US ranking in 24th,

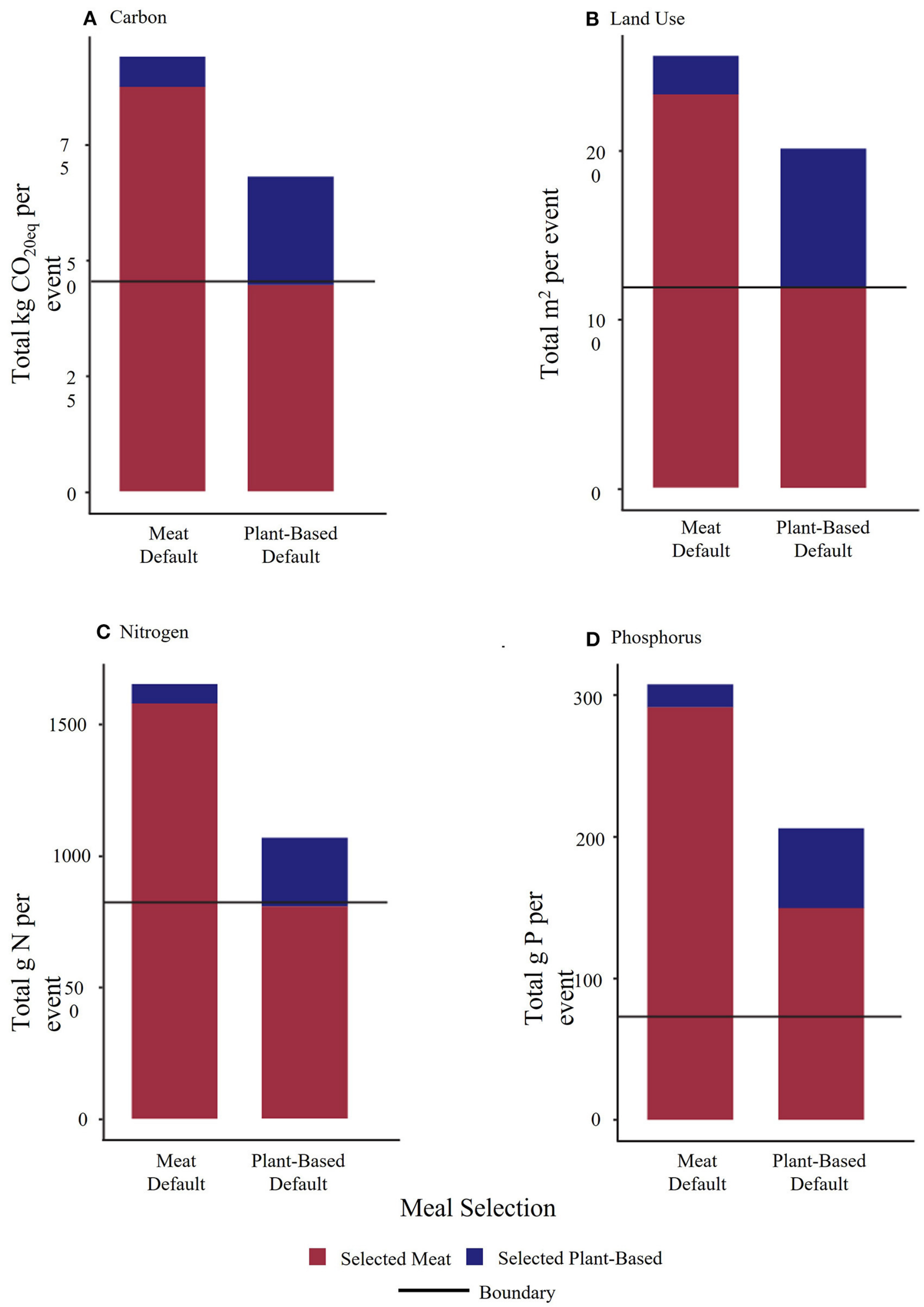

FIGURE 2
(A–D) Plant-based and chicken projected event calculations using the pooled RCTs. Colors represent breakdown of impacts from meat and veg meals. Black line represents EAT Lancet's planetary boundary for the measure (Willett et al., 2019).

27th and 26th in recent years (Hsu et al., 2016; Wendling et al., 2018, 2020).

Plant-based default interventions create positive effects for organizations, individuals, and the environment. The default intervention in our study managed to maintain participants' selections while reducing environmental impacts. Furthermore, a recent study revealed that plant-based and vegetarian dietary patterns in upper-middle-income countries were among the most affordable eating patterns (Springmann et al., 2021). As a result, organizations in the U.S using plant-based defaults could reduce environmental impact without additional cost while maintaining participant choice. Academic institutions such as UCLA, Harvard and many others have large catering services and hold regular catered events. According to the National Center for Education, 19.4 million students attended college in Fall 2020 (U. S. Department of Education, National Center for Education Statistics, 2021). If every student attended just one plant-based default catered event that year, a back of the envelope calculation estimates conservation of up to 27 million Kg (gigagram) of CO_2–carbon emissions from approximately 3 million gallons of gasoline (US Environmental Protection Agency, 2021). This calculation does not consider campus events held for faculty, staff, or industry professionals. Should a plant-based default nudge be implemented as a department-wide policy for catered events, planetary impacts could be reduced further. The concept of a default could also be applied beyond college campuses: hospitals, corporations, governments, and NGOs could also take steps to implement a plant-based default catering policy.

Environmental footprint

In the hypothetical 100-person events we modeled, we found that compared to using meat defaults, using plant-based or vegetarian defaults would reduce GHGEs, land use, phosphorus, and nitrogen by an estimated 38.9–42.7%. Implementing a plant-based or vegetarian default most improved projected impacts when the meat option was beef, but still led to substantial improvements when the meat option was chicken. Implementing a plant-based default improved projected impacts more than implementing a vegetarian default. Our results are consistent with research showing that plant-based food choices represent considerably lower environmental impacts as compared to animal-products of similar caloric content. Harwatt et al. (2017) found that substituting beans for beef could have achieved 46–74% of the reductions needed to meet the 2020 target for emissions in the US while also freeing up to 42% of cropland. A recent study modeled that rapidly phasing out animal agriculture has the potential to offset 25 gigatons of CO_2 and provide half the emissions reductions necessary for humanity to limit warming to 2°C (Eisen and Brown, 2022). Our research demonstrates that utilizing a plant-based default is a method that could help us phase out of our reliance on animal agriculture.

As seen in the single-meal calculations, the footprints of the vegetarian sandwich and the chicken sandwich were similar; so if the institution's primary objective for utilizing the default nudge is lowering the environmental footprint, a plant-based default over a vegetarian default would be the preferred choice (Table 3). The projected event with a bean and tofu sandwich as the default and a beef sandwich as the alternate option showed the greatest overall decrease in GHGE, land use, phosphorus, and nitrogen footprints compared to the situation when the default was reversed (Figures 3A–D). However, with regard to absolute footprint, serving the intervention group a plant-based meal default with a chicken meal option had the lowest environmental impact. This suggests that in the broad categories of "meat" (chicken or beef) and "plant-based" (plant-based or vegetarian), specific ingredients play a large role in determining impacts. In addition, the literature has shown that beef consumption in particular should be reduced due to its disproportionate impact on GHGEs, land use, nitrogen and phosphorus. Our results also highlight the outsized environmental effects of beef compared to other ingredients, and these findings align with other research. Beef production alone requires 28 times more land, 11 times more irrigation water, 5 times more greenhouse gas emissions, and 6 times more nitrogen than the average of dairy, poultry, pork or egg categories (Eshel et al., 2014).

Considering the environmental impacts exclusively, chicken is preferable to beef while legumes are preferable to protein from any animal source. However, because chickens are much smaller than cows, replacing beef with chicken dramatically exacerbates the negative animal welfare impact of meat consumption (Mathur, 2022). Additionally, chicken production can have other detrimental impacts, including proliferation of antibiotic resistance (Sanchez et al., 2020).

Despite significant differences in environmental impacts among the groups, each event footprint fell outside EAT-Lancet's established per-capita planetary boundaries (shown by the black lines in Figures 2A–D, 3A–D). This suggests we will need more than behavior change to fully move humanity within the planetary boundaries. Increasing food production efficiency in an equitable and sustainable manner will be necessary to limit land-use change, promote reduction as well as efficiency of nitrogen use, and encourage phosphorus recycling (Carpenter and Bennett, 2011; De Vries et al., 2013; Steffen et al., 2015; Campbell et al., 2017; Springmann et al., 2018a; Li et al., 2019). However, there are significant differences in the environmental impact of the same food items from different producers due to the diversities in agricultural practices. These differences provide opportunities to engage in both mitigation efforts at the producer level, as well as to educate consumers so they might make more environmentally friendly purchasing decisions (Poore and Nemecek, 2018).

Limitations

Our experiments were conducted at a sorority, a graduate student orientation, and an academic conference. Due to the geographical and cultural diversity of the U.S, more experiments should be conducted to determine the intervention's external validity. Regarding the limitations of the experiments themselves, one limitation is that demographic data was

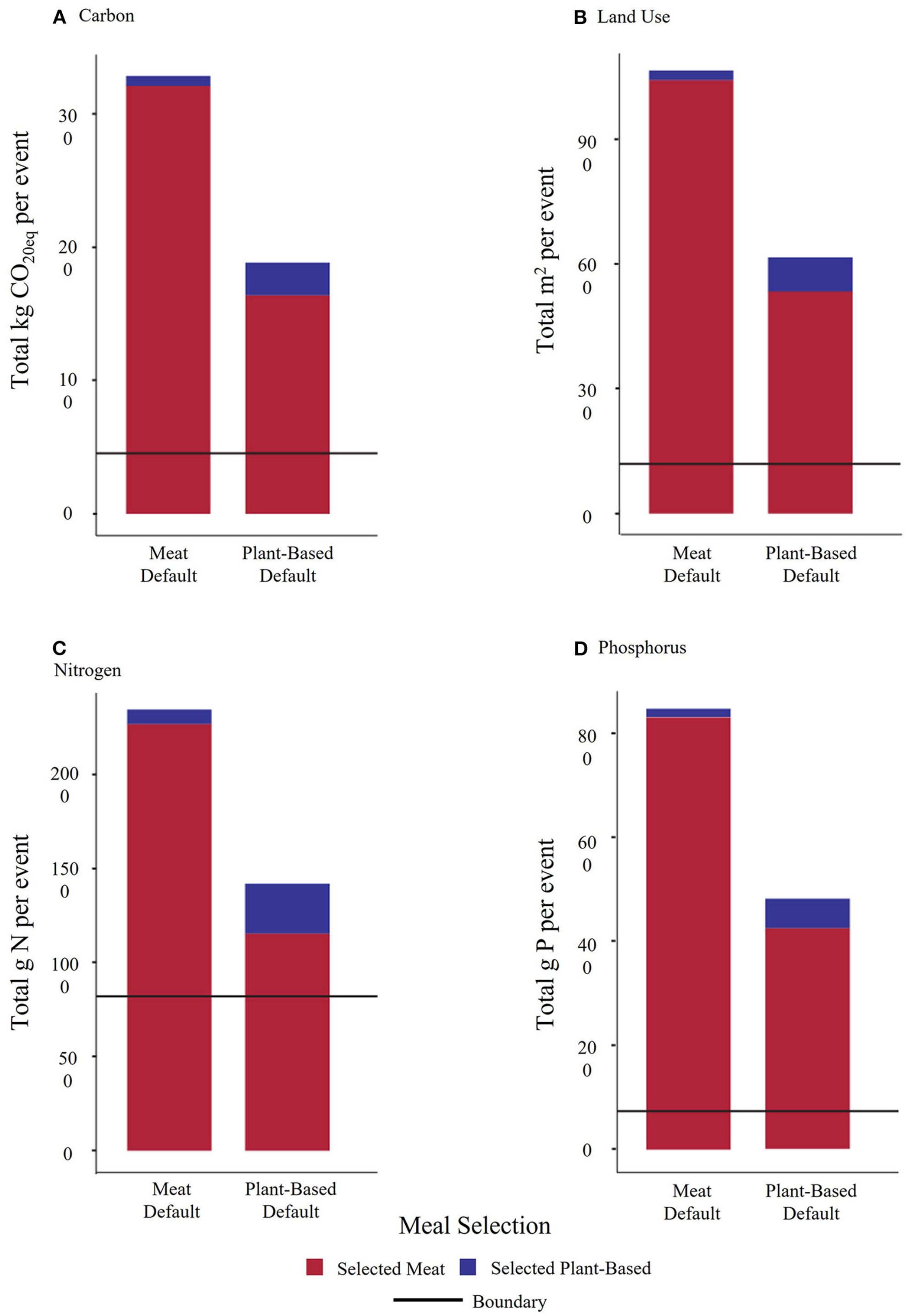

FIGURE 3
(A–D) Plant-based and beef projected event calculations using the pooled RCT results. Colors represent breakdown of impacts from meat and plant-based meals. Black line represents EAT-Lancet's planetary boundary for the measure (Willett et al., 2019).

not collected for the participants in this study. In planning this study there was concern that asking demographic questions to potential researchers (like those attending the BIH workshop or CEE graduate student orientation) would imply that they were participants in a study, thus influencing their responses. Demographic data was also considered inappropriate for the DG event given that the event was a sorority house dinner, where all attendees were well acquainted with the event operator.

Additionally, it was already known that the event would be composed of all female and female presenting participants. As a result, the lack of gender data in the CEE and BIH events, and homogeneity of genders for the DG event make it harder to disaggregate and evaluate the results of the intervention. Due to then attitudes tying meat consumption to masculinity among men (Love and Sulikowski, 2018; Nakagawa and Hart, 2019). Hansen et al. (2021) also found that women were more likely than men to remain with the default option when a plant-based meal was presented as the default.

Our study assesses the potential reductions in meat-based meals caused by using defaults to influence event participants' meal choices. The impact of food waste, food miles, and packaging were beyond the scope of our study but are required for a more accurate footprint of these events. Another limitation of our calculations is that the conversion factors used to calculate the environmental footprints are based on LCA data representing averages for food items. LCAs are created using assumptions and are ultimately simplified models for assessing an item's environmental impact (Curran, 2014). We also chose not to calculate the environmental impacts of processed foods that may typically be served due to limited available LCA data covering the complexity and variability of processed foods. The environmental impact associated with many processing methods has not yet been quantified.

We recognize the significance of the connection between health and nutrition when discussing the environmental footprints of meals. Despite this important link, our paper included no discussion on health due to prevalence of other available literature on this topic due to our focus on a single meal replacement. The EAT-Lancet report discusses health as a major priority. Therefore, if defaults are applied in other settings where they make a more significant contribution to overall caloric intake, their nutrition and health impacts should be seriously considered.

Suggestions for future research

In future work, more consideration should be made to implementation science and the barriers that event operators face in transitioning to a plant-based default. When implementing our experimental design, we found that willing event operators faced obstacles in providing delicious or varied plant-based options. Additionally, our calculations showed that specific food ingredients within broad meal categories (plant-based vs. vegetarian, beef vs. chicken) in part determined the efficacy of the intervention. Only by situating and understanding the intervention's implementation, barriers, and impacts in real contexts can we further expand institutional transitions to a plant-based default.

Future work on the default nudge for environmental purposes should test long-term efficacy and spillover effects. Previous studies investigating the default nudge have explored and shown positive effects of the intervention over time and partial persistence of behavior change after the intervention ended (Kurz, 2018). Additionally, a recent experiment on menu design for promoting sustainable food choices showed that people were more likely to choose vegetarian meals when the menu was at least 75% vegetarian (Parkin and Atwood, 2022). Future researchers could take Parkin and Atwood's findings a step further to test if the number of meal choices influence the selection of the plant-based default. This could serve to explain the discrepancy between our results and those found across the Hansen et al. events, which indicated the availability of a buffet.

Conclusion

In the U.S, few studies have explored nudging as a way to shift toward more sustainable dietary behaviors. To the authors' knowledge, this study is one of the first to do so in the context of higher education events. Furthermore, this research represents one of the few studies that quantify the potential environmental impacts of the default nudge using modeled menu choices but based on real-world food choice data. Based on our modeling, we found that the plant-based default nudge has the potential to reduce greenhouse gas, land-use, phosphorus and nitrogen footprints. We also find that specific ingredient types in broad meat and plant-based categorizations (i.e., chicken vs. beef) make a significant difference in determining event impacts.

At college campuses, adopting a campus-wide plant-based default policy can be an effective way to reduce environmental impact. A plant-based default could also be scaled and applied to other institutions beyond universities–such as corporate and government events, as well as in K-12 cafeterias. Announced September 28th, 2022, plant-based defaults have been implemented in three New York City hospitals (Mayor Adams Press Release, 2022). In January, 2022 the New York City school district also implemented a successful "Vegan Friday" program (Mayor Adams Press Release, 2022).

Our study, the established literature, and the implementation of programs like these demonstrate that people may not object to a higher proportion of plant-based meals, and that the nudge could be scaled to have a much larger impact than on catering alone. A plant-based default nudge policy could be implemented swiftly, without the use of expensive infrastructure or technology, all while maintaining participant choice. We demonstrate that this nudge has the potential to move us in the direction of a safe operating space for all.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by University of California Los Angeles Institutional Review Board and Harvard Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

RB: primary author of manuscript, made multiple revisions to draft, aided in design of study, and aided in data collection and analysis. AZ: primary author of manuscript, aided RB in making multiple revisions to draft, aided in design of study, analyzed RCT data and modeled environmental impact projections, and organized data collection. CS: wrote section in manuscript's introduction, provided expertise in design of the default intervention, and contributed edits to drafts of the manuscript. KP: aided in data collection and modeled environmental impact projections. MM: provided expertise on statistical methods and data analysis and contributed edits to drafts of the manuscript. DC: aided in design of study and contributed edits to drafts of the manuscript. DB and AM: led data collection at Harvard and reviewed manuscript. EG: aided in design of study and contributed expertise on choice architecture for sustainable behaviors. IB: aided in design of intervention and reviewed manuscript. MW: provided expertise on design of study. JJ: aided in design of study, contributed to writing of manuscript, aided in data analysis, edited multiple drafts of manuscript, and PI for project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The Better Food Foundation (where IB was employed) provided a grant to compensate AZ, a student on our project. Author MM is a member of the Research Advisory Boards of Greener by Default and of Sentience Institute. Author CS is also a member of the Research Advisory Boards of Greener By Default. Author IB is the Co-Director of the Greener by Default Advisory board. Author MW is a member of the Technical Advisory Committee for the Los Angeles County Food Equity Roundtable.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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SUPPLEMENTARY FILES

Supplementary Files: RSVP Surveys, Modeled meal recipes, and Environmental footprint sources.

References

Abadie, A., and Gay, S. (2006). The impact of presumed consent legislation on cadaveric organ donation: A cross-country study. *J. Health Econ.* 25, 599–620. doi: 10.1016/j.jhealeco.2006.01.003

Allen, A. M., and Hoff, A. R. (2019). Paying the price for the meat we eat. *Environ. Sci. Policy* 97, 90–94. doi: 10.1016/j.envsci.2019.04.010

Campbell, B. M., Beare, D. J., Bennett, E. M., Hall-Spencer, J. M., Ingram, J. S. I., Jaramillo, F., et al. (2017). Agriculture production as a major driver of the earth system exceeding planetary boundaries. *Ecol. Soc.* 22, 4. doi: 10.5751/ES-09595-220408

Campbell-Arvai, V., and Arvai, J., and Kalof, L. (2012). Motivating sustainable food choices: the role of nudges, value orientation, and information provision. *Environ. Behav.* 46, 453–475. doi: 10.1177/0013916512469099

Carpenter, S. R., and Bennett, E. M. (2011). Reconsideration of the planetary boundary for phosphorus. *Environ. Res. Lett.* 6, 014009. doi: 10.1088/1748-9326/6/1/014009

Carroll, G. D., Choi, J. J., Laibson, D., Madrain, B. C., Merick, A., et al. (2009). Optimal defaults and active decisions. *Q. J. Econ.* 124, 1639–1674. doi: 10.1162/qjec.2009.124.4.1639

Chinn, S. (2000). A simple method for converting an odds ratio to effect size for use in meta-analysis. *Statist. Med.*, 19, 3127–3131.doi: 10.1002/1097-0258(20001130)19:22<3127::aid-sim784>3.0.co;2-m

Clark, M. A., Domingo, N. G. G., Colgan, K., et al. (2020). Global food system emissions could preclude achieving the 1.5° and 2°C climate change targets. *Science* 370, 705–708. doi: 10.1126/science.aba7357

Curran, M. A. (2014). "Strengths and limitations of life cycle assessment," in Background and Future Prospects in Life Cycle Assessment, eds W. Klöpffer. Dordrecht: Springer Netherlands (LCA Compendium – The Complete World of Life Cycle Assessment), 189–206.

De Vries, W., Kros, J., Kroeze, C., Seitzinger, S. P., et al. (2013). Assessing planetary and regional nitrogen boundaries related to food security and adverse environmental impacts. *Curr. Opin. Environ. Sustain.* 5, 392–402. doi: 10.1016/j.cosust.2013.07.004

Eisen, M. B., and Brown, P. O. (2022). Rapid global phaseout of animal agriculture has the potential to stabilize greenhouse gas levels for 30 years and offset 68 percent of CO2 emissions this century. *PLoS Clim.* 1, e0000010. doi: 10.1371/journal.pclm.0000010

Eshel, G., Shepon, A., Makov, T., Milo, R., et al. (2014). Land, irrigation water, greenhouse gas, and reactive nitrogen burdens of meat, eggs, and dairy production in the United States. *Proc. Natl. Acad. Sci.* 111, 11996–12001. doi: 10.1073/pnas.1402183111

Garnett, E. E., Balmford, A., Sandbrook, C., Pilling, M. A., Marteau, T. M., et al. (2019). Impact of Increasing Vegetarian Availability on Meal Selection and Sales in Cafeterias. *Proc. Natl. Acad. Sci.* 116, 20923–20929. doi: 10.1073/pnas.1907207116

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., et al. (2013). "Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities," in *Tackling Climate Change Through Livestock: A Global Assessment of Emissions and Mitigation Opportunities*. Retrieved from: https://www.cabdirect.org/cabdirect/abstract/20133417883?q=(bn%3A%229789251079201%22) (accessed on March 3, 2022).

Godfray, H. C. J., and Oxford Martin School (2019). "Meat: the future series - alternative proteins," in *World Economic Forum*. Oxford University. Retrieved from: https://www.weforum.org/whitepapers/meat-the-future-series-alternative-proteins (accessed April 25, 2022.).

Hallström, E., Carlsson-Kanyama, A., and Börjesson, P. (2015). Environmental impact of dietary change: a systematic review. *J. Clean. Prod.* 91, 1–11. doi: 10.1016/j.jclepro.2014.12.008

Hansen, P. G., Schilling, M., and Malthesen, M. S. (2021). Nudging healthy and sustainable food choices: three randomized controlled field experiments using a vegetarian lunch-default as a normative signal. *J. Public Health* 43, 392–397. doi: 10.1093/pubmed/fdz154

Harwatt, H., Sabaté, J., Eshel, G., Soret, S., Ripple, W., et al. (2017). Substituting beans for beef as a contribution toward US climate change target. *Clim. Change* 143, 261–270. doi: 10.1007/s10584-017-1969-1

Herzog, H. (2014). "84% of vegetarians and vegans return to meat. Why?," in *Psychology Today*. Retrieved from: https://www.psychologytoday.com/us/blog/animals-and-us/201412/84-vegetarians-and-vegans-return-meat-why (accessed April 25, 2022).

Hsu, A., Esty, D. C., Levy, M. A., de Sherbinin, A., et al. (2016). *The 2016 Environmental Performance Index Report*. New Haven, CT: Yale Center for Environmental Law and Policy.

IPCC (2020). *Special Report on Climate Change and Land.* Available online at: https://www.ipcc.ch/srccl/ (accessed March 4, 2022).

Jachimowicz, J. M., Duncan, S. Weber, E. U., Johnson, E. J., et al. (2019). When and why defaults influence decisions: a meta-analysis of default effects. *Behav. Public Policy* 3, 159–186. doi: 10.1017/bpp.2018.43

Kleiber, C., and Zeileis, A. (2008). *Applied Econometrics with R*. Berlin, Germany: Springer-Verlag.

Kurz, V. (2018). Nudging to reduce meat consumption: Immediate and persistent effects of an intervention at a university restaurant. *J. Environ. Econ. Manage.* 90, 317–341. doi: 10.1016/j.jeem.2018.06.005

Li, M., Wiedmann, T., and Hdjkakou, M. (2019). Towards meaningful consumption-based planetary boundary indicators: The phosphorus exceedance footprint. *Glob. Environ. Chang.* 54, 227–238. doi: 10.1016/j.gloenvcha.2018.12.005

Love, H. J., and Sulikowski, D. (2018). Of meat and men: Sex differences in implicit and explicit attitudes toward meat. *Front. Psychol.* 9:559. doi: 10.3389/fpsyg.2018.00559

Mathur, M. B. (2022). Ethical drawbacks of sustainable meat choices. *Science* 375, 1362. doi: 10.1126/science.abo2535

Mayor Adams Press Release. (2022). *NYC H+H CEO Katz Announce Successful Rollout and Expansion of Plant-Based Meals as Default*. Available at: https://www1.nyc.gov/office-of-the-mayor/news/705-22/mayor-adams-nyc-h-h-ceo-katz-successful-rollout-expansion-plant-based-meals-as (accessed September 29th, 2022)

Meier, J., Andor, M. A., Doebbe, F., Haddaway, N., Reisch, L. A., et al. (2021). "Can Green Defaults Reduce Meat Consumption?," *SSRN*. Retrieved from: https://ssrn.com/abstract=3903160 (accessed on March 1, 2022).

Mertens, S., Herberz, M., Hahnel, U. J. J., Brosch, T., et al. (2022). The effectiveness of nudging: A meta-analysis of choice architecture interventions across behavioral domains. *Proc. Natl. Acad. Sci.* 119, e2107346118. doi: 10.1073/pnas.2107346118

Michalek, G., Meran, G., Schwarze, R., and Yildiz, Ö. (2015). "Nudging as a new 'soft' tool in environmental policy," in *An Analysis Based on Insights from Cognitive and Social psychology, No 21, Discussion Paper Series RECAP15, RECAP15* (Frankfurt: European University). Available online at: https://EconPapers.repec.org/RePEc:euv:dpaper:21 (accessed May 1, 2022).

Nakagawa, S., and Hart, C. (2019). Where's the beef? How masculinity exacerbates gender disparities in health behaviors. *Socius* 5, 2378023119831801. doi: 10.1177/2378023119831801

Nezlek. J. B., and Forestell, C. A. (2019). Vegetarianism as a social identity. *Curr. Opin. Food Sci.* 33, 45–51. doi: 10.1016/j.cofs.2019.12.005

Nykänen, E. P., Hoppu, U., Löyttyniemi, E., and Sandell, M. (2022). Nudging finnish adults into replacing red meat with plant-based protein via presenting foods as dish of the day and altering the dish sequence. *Nutrients* 14, 3973. doi: 10.3390/nu14193973

Ostfeld, R. J. (2017). Definition of a plant-based diet and overview of this special issue. *J Geriatr Cardiol.* 14, 315. doi: 10.11909/j.issn.1671-5411.2017.05.008

Parkin, B. L., and Atwood, S. (2022). Menu design approaches to promote sustainable vegetarian food choices when dining out. *J. Environ. Psychol.* 79, 101721. doi: 10.1016/j.jenvp.2021.101721

Perez-Cueto, F. J. A. (2021). Nudging plant-based meals through the menu. *Int. J. Gastron. Food Sci.* 24, 100346. doi: 10.1016/j.ijgfs.2021.100346

Pichert, D., and Katsikopoulos, K. V. (2008). Green defaults: information presentation and pro-environmental behaviour. *J. Environ. Psychol.* 28, 63–73. doi: 10.1016/j.jenvp.2007.09.004

Poore, J., and Nemecek, T. (2018). Reducing Food's Environmental Impacts Through Producers and Consumers. *Science* 360, 987–992. doi: 10.1126/science.aaq0216

R Core Team. (2022). *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing* (Vienna, Austria). Available online at: https://www.R-project.org/

Rare and the Behavioural Insights Team. (2019). *Behaviour Change for Nature: A behavioural toolkit for practioners*. Retrieved from: https://www.bi.team/publications/behavior-change-for-nature-a-behavioral-science-toolkit-for-practitioners/ (accessed March 1, 2022).

Sanchez, H. M., Whitener, V. A., Thulsiraj, V., Amundson, A., Collins, C., Duran-Gonzalez, M., et al. (2020). Antibiotic resistance of escherichia coli isolated from conventional, no antibiotics, and humane family owned retail broiler chicken meat. *Animals (Basel)* 10, 2217. doi: 10.3390/ani10122217

Scoditti, E., Tumolo, M. R., and Garbarino, S. (2022). Mediterranean diet on sleep, a health alliance. *Nutrients.* 14, 2998. doi: 10.3390/nu14142998

Springmann, M., Clark, M., Rayner, M., Scarborough, P., Webb, P., et al. (2021). The global and regional costs of healthy and sustainable dietary patterns: a modeling study. *Lancet Planet. Health* 5, e797–807. doi: 10.1016/S2542-5196(21)00251-5

Springmann, M., Wiebe, K., Mason-D'Croz, D., Sulser, T. B., Rayner, M., Scarborough, P., et al. (2018b). Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modeling analysis with country-level detail. *Lancet Planet. Health* 2:e451–61. doi: 10.1016/S2542-5196(18)30206-7

Springmann, M., and Clark, M., Mason-D'Croz, D., Wiebe, K., Bodirsky, B. L., Lassaletta, L., et al. (2018a). Options for keeping the food system within environmental limits. *Nature* 562, 519–525. doi: 10.1038/s41586-018-0594-0

Stapleton, J. (2009). *Linear Statistical Models*. Hoboken, N.J: Wiley.

Steffen, W., Richardson, K., Rockström, J., Cornell, S. E., Fetzer, I., Bennett, E. M., et al. (2015). Planetary boundaries: guiding human development on a changing planet. *Science* 347, 1259855. doi: 10.1126/science.1259855

Thaler, R. H., and Benartzi, S. (2004). Save More Tomorrow^{TM}: Using Behavioral Economics to Increase Employee Saving. *J. Pol. Econ.* 112, S164–87. doi: 10.1086/380085

Thaler, R. H., and Sunstein, C. R. (2008). *Nudge: Improving Decisions About Health, Wealth, and Happiness*. New Haven, CT: Yale University Press.

Tzivia, M., Negro, S. O., Kalfagianni, A., Hkkert, M. P., et al. (2020). Understanding the protein transitions: the rise of plant-based meat substitutes. *Environ. Innov. Soc. Trans.* 35, 217–231. doi: 10.1016/j.eist.2019.09.004

US Environmental Protection Agency (2021). *EPA Automotive Trends Report. (Data file)*. Retrieved from: http://www.epa.gov/automotive-trends/explore-automotive-trends-data (accessed April 20, 2022).

U. S. Department of Education, National Center for Education Statistics. (2021). *IPEDS. (Data file)*. Retrieved from: https://nces.ed.gov/ipeds/search/ViewTable?tableId=29448 (accessed April, 20, 2022).

Van Gestel, L. C., Adriaanse, M. A., and De Ridder, D. T. D. (2020). Do Nudges Make Use of Automatic Processing? Unraveling the Effects of a Default Nudge under Type one and Type 2 Processing. *Compreh. Results Soc. Psychol.* 0, 1–21. doi: 10.1080/23743603.2020.1808456

Vandenbroele, J., Vermeir, I., Geuens, M., Slabbink, H., and Van Kerckhove, A. (2019). Nudging to get our food choices on a sustainable track. *Proc. Nutr. Soc.* 79, 133–146. doi: 10.1017/S0029665119000971

VanderWeele, T. J. (2020). Optimal approximate conversions of odds ratios and hazard ratios to risk ratios. *Biometrics* 76, 746–752. doi: 10.1111/biom.13197

Vecchio, R., and Cavallo, C. (2019). Increasing healthy food choices through nudges: a systematic review. *Food Quali Pref.* 78, 103714. doi: 10.1016/j.foodqual.2019.05.014

Vizcaino, M., Ruehlman, L. S., Karoly, P., Shilling, K., Berardy, A., Lines, S., et al. (2020). A goal-systems perspective on plant-based eating: keys to successful adherence in university students. *Public Health Nutr.* 24, 75–83. doi: 10.1017/S1368980020000695

Wendling, Z. A., Emerson, J. W., de Sherbinin, A., Wolf, M. J., Esty, D. C., Mangalmurti, D., et al. (2020). "Environmental performance index," in *Yale Center Environmental Law and Policy*. Available online at: https://epi.envirocenter.yale.edu/node/36476

Wendling, Z. A., Esty, D. C., Emerson. J. W., Levy, M. A., de Sherbinin, A., Spiegel, N. R. et al. (2018). "The 2018 environmental performance index report," *Yale Center for Environmental Law and Policy*.

White, H. (1980). A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica* 48, 817. doi: 10.2307/1912934

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the anthropocene: the EAT–lancet commission on healthy diets from sustainable food systems. *Lancet* 393, 447–492. doi: 10.1016/S0140-6736(18)31788-4

Zeileis, A., Köll, S., and Graham, N. (2020). Various versatile variances: an object-oriented implementation of clustered covariances in R. *J. Stat. Softw.* 95, 1–36. doi: 10.18637/jss.v095.i01

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Impact of aging on food consumption in rural China: Implications for dietary upgrading and health improvement

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Background: The issue of population aging in rural China is getting profound; nevertheless, its impact on food consumption has not been well evaluated. This study aims to examine the relationship between rural aging and family food consumption in rural China.

Materials and methods: Using the statistical yearbook data and the nationally representative household-level data from the China Rural Fixed Observation Points, this study compares the evolution of food consumption between rural and urban residents from 1985 to 2020 and analyzes the structure of food consumption expenditure of rural residents. Next, this study further investigates the impact of aging on food consumption in rural households with ordinary least squares.

Results: (1) The principal foods consumed by rural residents in 2020 are meat and meat products (36.8%), grain (24.5%), and vegetables (10.9%). (2) An increase in older adults has decreased the absolute consumption of all foods, while it increased relative consumption of meat and meat products, aquatic products, edible oil and fats, poultry, eggs, and sugar. (3) Due to differences in the structure of young adults' food consumption, older adults would increase their consumption of fruits and vegetables if they lived with younger adults.

Conclusion: The findings of this study suggest that rural older adults may increase their consumption of fruits and vegetables by advocating intergenerational cohabitation while maintaining their intake of protein to achieve a balanced dietary structure and improve their health condition.

KEYWORDS

aging, food consumption, intergenerational cohabitation, household composition, health improvement

Introduction

Reasonable food consumption and nutrition intake are basic guarantees of physical health and high-quality human capital, which are of great importance to increasing labor productivity and promoting long-term social development (1). Combined with steady growth in income level, the total food consumption of Chinese residents has significantly increased (2). In contrast to food consumption in China in 1978, the consumption of cereals, meat, dairy products, and vegetables and fruits in 2015 reached 455.2, 91.8, 51.1, and 553 million tons, respectively, which represented increases of 220, 730, 1,610, and 950%, respectively (3). Rural residents have a lower intake of protein and vitamins and an excessive intake of staple foods compared with urban residents (4). By the end of 2020, there were still 510 million rural residents, accounting for 36.11% of the total population of China (5), indicating that the issue of food consumption by rural residents cannot be ignored.

Meanwhile, China is facing severe aging challenges; according to the Seventh National Census in 2020, the number of people aged over 60 years reached 264 million (18.70%). The issue of aging in rural areas is even more severe, and the proportion of older adults in rural areas is 23.81%, which is much higher than that in urban areas (6). The rapid increase in the proportion of older adults in rural China has profoundly impacted rural residents' food consumption (7). Noticeably, as aging continues to be a serious problem in rural areas (8), the rise in the proportion of older adults may have been an increasingly important factor that affects food consumption in rural China.

Generally, there are usually two types of influencing factors that affect food consumption: macrolevel factors, such as agricultural production, economic growth, urbanization, and infrastructure construction (9–12); and microlevel factors, such as education level, household income, family structure, food prices, and social and psychological traits (2, 13–16). There is also a small section of the literature that focuses on the impact of aging on food consumption. Considering the health condition of older adults, less physical activity and degenerated body organs usually lead to a decline in total food consumption (17, 18). There is empirical evidence that accelerated aging may decrease food consumption per capita (19). However, a decrease in total food consumption does not mean a decrease in each type of food consumption. Although rural older adults choose to decrease meat consumption to some extent (20), they also consciously prefer to consume healthier foods to obtain more nutrients (21–23). In addition, older adults' gender, income level, personal traits (24), and family structure are also important factors affecting food consumption (25–29).

From the above literature review, the following gaps require more attention. First, most of the aforementioned literature only considers the impact of aging at the regional level, which may lack the analysis of national micro household survey data. Second, there seems to be less research on the impact of aging on food consumption by rural residents, with most related studies paying attention to Chinese residents or urban residents. Finally, there is little research on how aging in rural China affects specific food consumption at the household level. Most available studies pay more attention to the consumption of staple foods and meat, ignoring aquatic products, dairy products, fruits, and vegetables. However, these types of foods are also an important part of rural residents' food consumption.

This study aims to explain how aging affects family food consumption structures and expenditures in rural families, thereby providing empirical evidence and policy implications for the improvement of diet and health of rural residents. Additionally, this study seeks to fill various research gaps. We use nationally representative household survey data to study the impacts of older adults on food consumption, which makes the conclusions more reliable. We divide food consumption into 10 categories, which helps us understand the impact of aging on specific food consumption more accurately. This study also considers the impact of young and older adults living together, which complements the analysis of the external effect of demographic shifts on households. Considering older adults' low vitamin intake, this study puts forward the beneficial countermeasures that older adults should increase expenditure on fruits and vegetables and maintain the intake of protein to improve their dietary structure and enhance physical fitness.

Materials and methods

Data

In our study, we use two types of data. The first type of data is from the National Household Income and Expenditure Survey, which is carried out by the National Bureau of Statistics of the Chinese Government. National Household Income and Expenditure Survey adopted a stratified multi-stage random sampling method and included 160,000 urban and rural households from 31 provinces of China. The survey includes detailed records about household level food consumption. The National Bureau of Statistics aggregated household level data, conducted weighted statistics, and then released the annual report about the average food consumption of urban and rural residents. Notably, the micro household data are not publicly available, and we could only obtain information about the aggregated average food consumption of urban and rural residents from the annual report. More specifically, four types of food consumption per capita of urban and rural residents from 1985 to 2020 are used for the analysis of rural–urban residents' food consumption in section "The evolution of rural-urban residents' food consumption".

The second type of data used in this study was retrieved from the China Rural Fixed Observation Points in 2020, collected by the Research Center for Rural Economy under the Ministry of Agricultural and Rural Affairs of China, which includes comprehensive information about rural households and their members. This survey data only target rural households, not urban households, which is used to analyze the structure of food consumption of rural households in section "The structure of food consumption expenditure in rural households" and the regressions on the effects of rural aging on food consumption in sections "Regression of food consumption per capita on aging" and "Regression of food consumption structure on aging". The survey was formally set up in 1986 and has been run until now, covering 23,000 rural households and 375 villages in 368 counties of 31 provinces. It is nationally representative survey data. Equal survey weight is given to poor, medium, and rich rural families in typical counties of each province in China. These data have been widely used in academic research (30–33). The data consist of 1,250 indicators in eight categories, including labor force, annual household income, and consumption. Notably, detailed information regarding the consumption of various foods by rural households is available in 2020, which builds a solid foundation for this research. Thus, we only use survey data from 2020, and the final sample size for the empirical analysis was 20,185 after removing observations with missing values.

TABLE 1 Descriptive statistics of continuous variables (N = 20,185).

Variables	Mean	Std. dev.
Per capita consumption expenditure of grain (yuan)	24.5	14.76
Per capita consumption expenditure of edible oil and fats (yuan)	9.0	6.15
Per capita consumption expenditure of vegetables and edible mushrooms (yuan)	10.9	8.53
Per capita consumption expenditure of meat and meat products (yuan)	36.8	15.02
Per capita consumption expenditure of poultry (yuan)	3.3	4.09
Per capita consumption expenditure of aquatic products (yuan)	4.3	4.73
Per capita consumption expenditure of eggs (yuan)	3.2	3.14
Per capita consumption expenditure of milk and dairy products (yuan)	2.7	4.11
Per capita consumption expenditure of melons and fruits (yuan)	4.7	4.41
Per capita consumption expenditure of sugar (yuan)	0.7	0.83
Weighted mean age (years)	39.7	18.64
Household income (10,000 yuan)	8.4	7.04
Proportion of labor force (%)	65.0	30.38

1 RMB (Yuan) = 0.14493 USD (dollar) in 2020.

Measurement

We focused on two dependent variables: (1) the food consumption expenditure per capita on a specific food (unit: yuan) and (2) the proportion of the consumption expenditure on a specific food in total expenditure (unit:%). These two variables reflected the quantity and structure of food consumption among rural residents. Notably, we obtained information on family food expenditures and family members from the Household Questionnaire. The Household Questionnaire is generally answered by the head of the household. Heads of households usually have accurate information about family members and food consumption and provide clear answers, guaranteeing high-quality data. Thus, per capita expenditure is accurate. The detailed food items can be found in **Supplementary Table 1**.

Based on the classification of food consumption by the National Bureau of Statistics of China, 10 main types of food were selected: (1) grains, (2) edible oil and fats, (3) vegetables and edible mushrooms, (4) meat and meat products, (5) poultry, (6) aquatic products, (7) eggs, (8) milk and dairy products, (9) melons and fruits, and (10) sugar.

Additionally, we focused on two core independent variables: (1) the number of people in the household aged 65 years and over (NP65) and (2) the proportion of people aged 65 years and over (PP65). These two independent variables were treated as follows: (1) NP65 was transformed into a categorical variable with values of 0, 1, and 2, where 2 indicates the number of older adults equal to or more than 2. There were 57.1% of rural households with no older adults, 21.4% of rural households with one older adult, and 21.5% of rural households with two or more older adults. (2) For PP65, we defined three types of rural households: pure young family (PP65 = 0), mixed family (0 < PP65 < 1), and pure older adult family (PP65 = 1). The proportions of pure young, mixed, and pure older adult families were 57.1, 13.4, and 29.4%, respectively.

We excluded the confounding influence of wealth (34), workforce composition (35), and other demographic characteristics at the household level by adding the following controlling variables: mean age of household members weighted by the length of stay at home, which is used for controlling for the interference of family member's age; whether the household was poor, and the proportion of poor families was 7.4%, which is used for controlling family wealth status; annual household income because household income level directly determines the quantity and quality of food consumption (36); and the proportion of labor force, which may affect family food consumption patterns (37). The descriptive statistics of relative variables can be seen in **Table 1**.

Analytical strategy

This study's analysis was conducted in two parts. First, we performed a descriptive statistical analysis of rural and urban residents' food consumption evolution with the aggregated data of the National Household Income and Expenditure Survey, which aimed to identify the consumption variation in four main food types of rural and urban residents. Due to the data availability, we cannot conduct statistical tests with household data, but only conduct descriptive statistics with aggregated data. We also describe the composition of food consumption expenditure in rural households with the micro data of China Rural Fixed Observation Points in 2020 in section "The structure of food consumption expenditure in rural households".

Second, we used ordinary least squares (OLS) to empirically analyze the relationship between aging and food consumption in rural households (38) with the micro data of China Rural Fixed Observation Points in 2020. There are four analyses: the relationship between the number of older adults and the food consumption per capita, the relationship between household composition and food consumption per capita, the relationship between the number of older adults and the structure of food consumption, and the relationship between household composition and the structure of food consumption.

Results

Trends and composition of food consumption

In this section, we first analyze the evolution of food consumption of grains, pork, aquatic products, and edible vegetable oil separately from 1985 to 2020 among rural and urban residents; and second, we concentrate on the structure of food consumption expenditure of rural residents in detail with the latest data from unique nationally representative household survey data.

The evolution of rural–urban residents' food consumption

From Figure 1A, the grain consumption gap between urban and rural residents has narrowed continuously, from 122.7 kg per capita in 1985 to 48.2 kg per capita in 2020. However, there is still a noticeable gap of 48.2 kg per capita.

From Figure 1B, Chinese residents' edible vegetable oil consumption shows a trend of fluctuating growth. Usually, rural residents' edible vegetable oil consumption is significantly lower than that of urban residents. However, this was the first time that the consumption of edible vegetable oil by rural residents surpassed that of urban residents in 2018.

From Figure 1C, the pork consumption of rural residents caught up with that of urban residents from 1985 to 2020. Noticeably, in 2018, rural residents' pork consumption (23 kg per capita) surpassed urban residents' pork consumption (22.7 kg per capita), showing the rapidly growing demand of rural residents for pork. Simultaneously, the pork consumption of rural and urban residents seems to have peaked at 20 kg per capita.

From Figure 1D, the strong growth in aquatic product consumption is indicated by an increase of 235.21% and 643.75% for urban and rural residents, respectively. In the 1980s, the aquatic product consumption of urban and rural residents was 7.1 and 1.6 kg per capita, respectively. In recent years, the growth rate of aquatic products for rural residents has been much higher than that of urban residents. Although the urban–rural gap in aquatic product consumption has greatly decreased over the past 35 years, there is still a disparity.

The structure of food consumption expenditure in rural households

We also discuss the latest rural households' food consumption expenditures using survey data from 2020. In Figure 2, there are 10 main types of food in the rural residents' food list. Rural residents' food consumption structure has gradually transformed from a single to a diversified structure. From the perspective of food composition, meat and meat product consumption was ranked first, accounting for more than one-third of total food expenses. The proportion of grain consumption was 24.52% and ranked second. The expenditure on vegetables and edible mushrooms approximately equals that of edible oil and fats, accounting for 10% of the total cost. However, aquatic product, egg, milk, and dairy product expenditures still represent a small proportion, adding up to only 10%, indicating that rural residents' protein intake may be insufficient and the quality of food consumption needs further improvement.

Regression of food consumption per capita on aging

From Tables 2, 3, we find that food consumption per capita is related to the number and proportion of older adults in rural households.

Regression of food consumption per capita on the number of older adults

Table 2 indicates that an increase in the number of older adults significantly lowers the consumption of all 10 types of food. With the increase in older adults, the decline in spending on meat and meat products and grains ranked first and second, respectively. Compared with the family with no older adults, the consumption of meat and meat products and

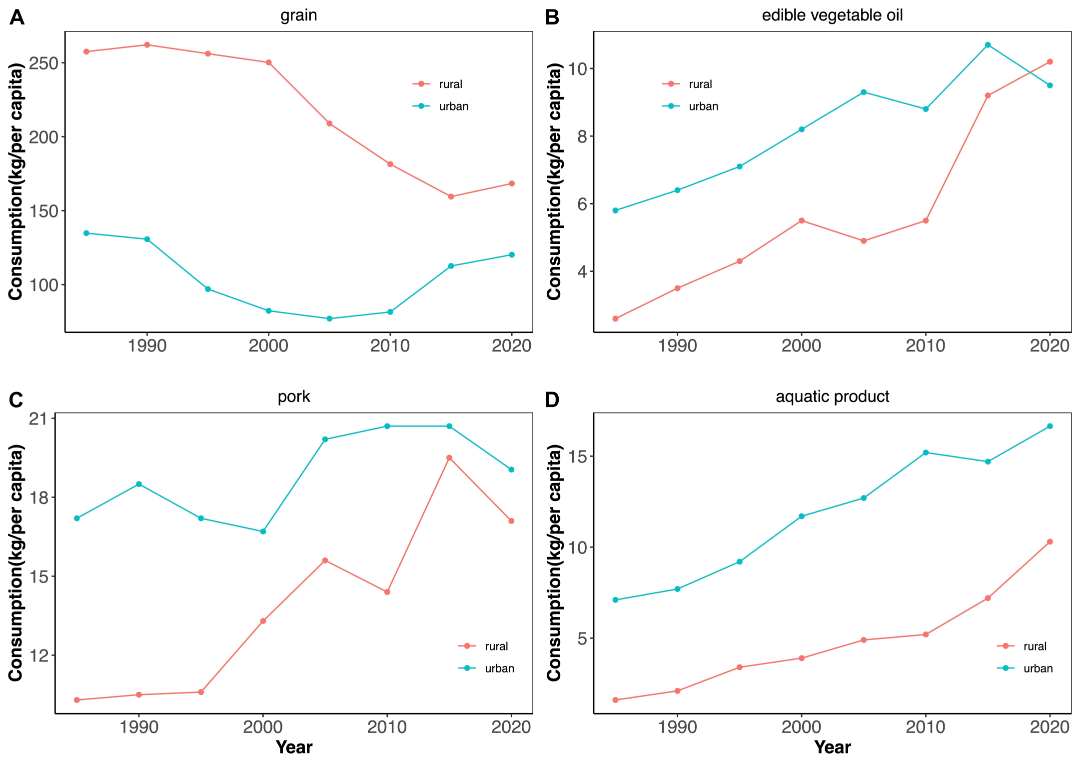

FIGURE 1
Urban-rural trends of per capita consumption of four main foods from 1985 to 2020. The data has been aggregated to the urban and rural average level from the micro household level by the National Bureau of Statistics. The occasion of observation is equal to 36 (More specifically, 36 for the average food consumption of rural residents from 1985 to 2020, and 36 for the average food consumption of urban residents in each year from 1985 to 2020). The red and blue lines denote per capita consumption of grain, edible vegetable oil, pork, and aquatic product of rural and urban residents, respectively.

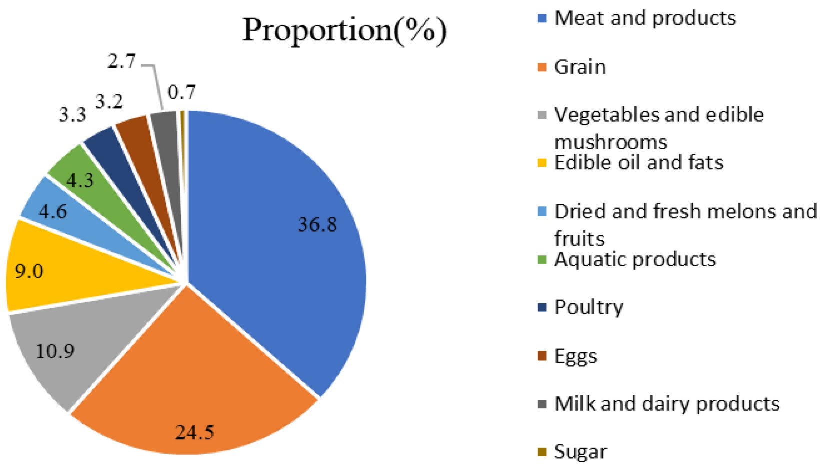

FIGURE 2
Rural residents' food consumption expenditure structure in 2020, unit: %.

grain in the family with one older adult decreased by 67 and 54 yuan, respectively. In the case of a family with two or more older adults, the decrease in meat and meat products and grains was 178 and 109 yuan, respectively. Compared with the family with no older adults, spending on vegetables and edible mushrooms, edible oil and fats, and melons and fruits in the family with one older adult decreased by 32, 10, and 22 yuan, respectively. However, the spending on these three types of

TABLE 2 Regression of the food consumption per capita on the number of older adults.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Variables	**Grain**	**Edible oil and fats**	**Vegetables and edible mushrooms**	**Meat and meat products**	**Poultry**	**Aquatic products**	**Eggs**	**Milk and dairy products**	**Melons and fruits**	**Sugar**
Zero old = ref.										
one old	−53.76***	−10.43***	−31.71***	−67.09***	−5.686**	−9.979***	−5.843***	−4.689***	−21.93***	−1.248***
	(6.069)	(2.419)	(5.159)	(11.78)	(2.255)	(2.604)	(1.228)	(1.615)	(2.070)	(0.252)
two old	−108.6***	−34.64***	−86.77***	−177.8***	−17.59***	−21.62***	−13.22***	−12.83***	−30.61***	−3.016***
	(6.668)	(2.658)	(5.669)	(12.94)	(2.478)	(2.862)	(1.349)	(1.774)	(2.274)	(0.277)
R-squared	0.051	0.036	0.036	0.040	0.027	0.057	0.038	0.028	0.044	0.035

**$p < 0.05$; ***$p < 0.01$. N = 20,185. Covariates include weighted mean age, household income, poverty status, and proportion of labor force (%).

TABLE 3 Regression of the food consumption per capita on the household composition.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Variables	**Grain**	**Edible oil and fats**	**Vegetables and edible mushrooms**	**Meat and meat products**	**Poultry**	**Aquatic products**	**Eggs**	**Milk and dairy products**	**Melons and fruits**	**Sugar**
Young household = ref.										
Mixed household	−99.30***	−27.15***	−65.32***	−140.6***	−15.88***	−22.27***	−13.58***	−11.68***	−29.14***	−2.467***
	(5.436)	(2.175)	(4.649)	(10.60)	(2.027)	(2.337)	(1.099)	(1.452)	(1.863)	(0.227)
Old household	48.91***	15.88***	3.455	36.89**	18.32***	26.49***	16.98***	11.83***	−5.726*	0.668*
	(8.888)	(3.556)	(7.601)	(17.34)	(3.315)	(3.822)	(1.796)	(2.374)	(3.046)	(0.372)
R-squared	0.062	0.040	0.037	0.042	0.032	0.065	0.051	0.033	0.047	0.037

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$. N = 20,185. Covariates include weighted mean age, household income, poverty status, and proportion of labor force (%).

food for those families with two or more older adults decreases by 87, 35, and 31 yuan, respectively. In addition, it is worth noting that there is a smaller decrease in the consumption of aquatic products, poultry, eggs, milk and dairy products, and sugar.

Regression of food consumption per capita on household composition

Table 3 shows that the food consumption of mixed families is significantly lower than that of young families. Specifically, food consumption per capita on meat and meat products and grains in mixed families is 140 and 99 yuan lower than in young families, which is the main difference between these two types of families. The gaps in vegetables and edible mushrooms, edible oil and fats, and melons and fruits are 65, 27, and 29 yuan, respectively. Furthermore, the gaps in poultry, aquatic products, eggs, milk and dairy products, and sugar are relatively small, at no more than 25 yuan. Noticeably, food consumption per capita in pure older adult families is significantly higher than that in young families. The most obvious gaps between young and older adult families are reflected in the consumption of grain and meat and meat products, at 49 and 37 yuan, respectively.

Regression of food consumption structure on aging

Next, we analyze the impact of the number and proportion of older adults on the structure of food consumption at the household level.

Regression of food consumption structure on the number of older adults

Table 4 shows the regression results of the proportion of the consumption of various foods on the number of older adults. With an increase in the number of older adults, the variation in different types of foods is the opposite. First, the proportion of grains, vegetables, and edible mushrooms and melons and fruits declined, which is consistent with the variation in corresponding food consumption. Compared with a family with no older adults, having one or two older adults in the family reduces the proportion of grain consumption by 1.0 or 1.2 percentage points, while the decrease was 0.1 and 0.8 percentage points for vegetables and edible mushrooms, respectively, and 0.5 and 0.4 percentage points for melons and fruits, respectively. Second, the proportion of consumption of edible oil and fats, meat and meat products, poultry, aquatic products, eggs, milk

TABLE 4 Regression of the proportion of food consumption (%) on the number of older adults.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Variables	**Grain**	**Edible oil and fats**	**Vegetables and edible mushrooms**	**Meat and meat products**	**Poultry**	**Aquatic products**	**Eggs**	**Milk and dairy products**	**Melons and fruits**	**Sugar**
Zero old = ref.										
one old	−0.972***	0.333***	−0.110	0.979***	0.0990	0.0805	−0.0364	0.109	−0.496***	0.0153
	(0.281)	(0.117)	(0.162)	(0.287)	(0.0777)	(0.0889)	(0.0600)	(0.0785)	(0.0840)	(0.0157)
two old	−1.204***	0.249*	−0.827***	1.255***	0.171**	0.447***	0.131**	0.134	−0.394***	0.0391**
	(0.309)	(0.129)	(0.179)	(0.316)	(0.0854)	(0.0977)	(0.0660)	(0.0863)	(0.0923)	(0.0173)
R-squared	0.015	0.011	0.014	0.006	0.020	0.040	0.007	0.008	0.014	0.011

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$. N = 20,185. Covariates include weighted mean age, household income, poverty status, and proportion of labor force (%).

TABLE 5 Regression of the proportion of food consumption (%) on the household composition.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Variables	**Grain**	**Edible oil and fats**	**Vegetables and edible mushrooms**	**Meat and meat products**	**Poultry**	**Aquatic products**	**Eggs**	**Milk and dairy products**	**Melons and fruits**	**Sugar**
Young household = ref.										
Mixed household	1.287***	0.340***	−0.339**	1.379***	0.0922	0.155*	−0.0513	0.0731	−0.393***	0.0311**
	(0.253)	(0.106)	(0.146)	(0.259)	(0.0700)	(0.0801)	(0.0541)	(0.0708)	(0.0757)	(0.0142)
Old household	0.144	0.0616	−0.822***	−0.493	0.336***	0.682***	0.512***	0.378***	−0.791***	−0.00671
	(0.414)	(0.173)	(0.239)	(0.423)	(0.115)	(0.131)	(0.0884)	(0.116)	(0.124)	(0.0232)
R-squared	0.016	0.011	0.013	0.007	0.020	0.040	0.009	0.008	0.015	0.011

*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$. N = 20,185. Covariates include weighted mean age, household income, poverty status, and proportion of labor force (%).

and dairy products, and sugar increased, even though there are more older adults in the household. Among these types of food, the proportion of meat and meat product consumption increased the most by 1 and 1.3 percentage points, respectively. In addition, in a family with two older adults, the increase in the proportion of food consumption of edible oil and fats, poultry, aquatic products, eggs, and sugar was 0.25, 0.2, 0.5, 0.1, and 0.04 percentage points, respectively.

Regression of food consumption structure on household composition

From the perspective of family types (such as young, mixed, and older adult families), we can see the structure of food consumption from Table 5. Based on the results in this table, we classify the impact of the proportion of older adults into three categories. First, for vegetables and edible mushrooms, and melons and fruits, the proportion of consumption of vegetables and edible mushrooms in mixed and older adult families is 0.3 and 0.8 percentage points lower than that in young families, and the proportion of spending on melons and fruits is 0.4 and 0.8 percentage points lower. Thus, due to differences in the structure of young adults' food consumption, older adults would increase their consumption of fruits and vegetables if they lived with younger adults.

Second, there were significant differences in the proportion of consumption of poultry, aquatic products, eggs, and milk and dairy products between older adults and young families. Compared with young families, the proportion of older adult families' consumption of these four types of food is 0.3, 0.7, 0.5, and 0.4 percentage points lower, respectively.

Third, there are observable differences in the consumption of grain, edible oil and fats, meat and meat products, and sugar between mixed and young families. The proportion of food consumption of these types of food in the mixed families was 1.3, 0.3, 1.4, and 0.03 percentage points higher than that in the young families.

Discussion and conclusion

This study described the food consumption trends of rural and urban residents from 1985 to 2020 and the structure of food consumption of rural residents in China in 2020. Since 1985, grain consumption per capita in rural areas has decreased gradually. However, there is still one noticeable difference in grain consumption between rural and urban residents (4). The growth rate of aquatic product consumption among rural residents was faster than that among urban residents. However, the consumption of edible oil and fats, and pork per capita in rural areas was equal to that in urban areas, which shows the huge demand for meat and meat products and oil in rural China. By 2020, the proportion of food consumption of both meat and meat products and grain in the total food consumption accounted for 61%. This result shows that rural residents' food consumption tends to be diverse (39). Along with the increase in the supply of various types of food, rural residents' demand for high-quality food has been satisfied to some extent (40).

Next, we studied the relationship between aging and food consumption in rural China. In 2020, Engel's coefficient of rural residents was approximately 32.7%, which is not in line with the actual situation in rural residents' living levels (41). Thus, we pay attention not only to how much food rural residents consume but also to what kind of food they consume. At the household level, the presence of older adults may significantly affect the structure of food consumption. This study focuses on the impact of the number of older adults and household composition on the amount and structure of food consumption at the household level.

On one hand, an increase in the number of older adults improves relative food consumption of edible oil and fats, poultry, aquatic products, eggs, milk and dairy products, and sugar. In addition, food consumption per capita would increase if all family members were older adults. This may be related to the amount of time that older adults spend at home. Generally, older adults spend more time at home, whereas young adults spend less time there (42). Therefore, the food consumption per capita in pure older adult families is greater than that in pure young families. As mixed families are usually made up of three generations and children usually have lunch at school, food consumption per capita in mixed families is the lowest. Noticeably, there is an interesting phenomenon that, compared with young people, older adults consume less of melons and fruits, which is consistent with the previous literature (43). Thus, the fruit consumption of both pure older adult families and mixed families is lower than that of pure young families. This suggests that older adults have a lower preference for fruit. Although such products may be unregistered in rural areas due to their own production and consumption, rural residents' consumption of fruit and vegetables is still relatively low compared with urban residents' consumption (44).

On the other hand, we found empirical evidence that intergenerational cohabitation may affect the structure of food consumption. Importantly, older adults increase their consumption of fruits and vegetables if they live with younger adults. There are obvious similarities and differences in the structure of food consumption between older and younger people (45). First, regarding the proportion of total consumption of grain, edible oil and fats, meat and meat products, and sugar, older adults and younger people show similar food consumption structures. However, when older adults and young people live together, the aforementioned food consumption structure shows variations with different characteristics. Second, there are significant differences in food consumption between young and older adults in terms of poultry, aquatic products, eggs, milk and dairy products, vegetables and edible mushrooms, and melons and fruits (46). When they live together, the differences become narrow or even disappear.

This study may contribute to the following aspects. First, we find that the number of older adults and household composition significantly affect the amount and structure of food consumption in Chinese rural households. Second, this study focuses on analyzing the impact of intergenerational cohabitation, such as older adults and young people living together on the structure of food consumption. Specifically, this study complements the analysis of the second demographic transition in China and concludes that there are pronounced differences in food consumption among various family types, such as in pure older adult, mixed, and pure young families.

This study draws the following main conclusions. First, since the reform and opening up, food consumption of Chinese rural residents has tended to be diversified, consistent with previous studies (39). Second, this study provides empirical evidence that aging significantly affects the food consumption of rural residents. More specifically, food consumption per capita is related to the number of older adults and household composition in rural households. Third, older and younger family members show different preferences regarding food consumption, and intergenerational cohabitation may significantly affect the structure of family food consumption.

The conclusions of this study could provide a decision-making basis for promoting food consumption diversification and improving the quality of food consumption by older adults in rural China. To ensure the balance of dietary structure and health improvement of rural older adults, we suggest that rural older adults should increase their consumption of fruits and vegetables by advocating intergenerational cohabitation and maintain a sufficient intake of protein by appropriately increasing the consumption of high-protein beef and fish.

It is worth noting that this study may have the following limitations due to the restriction of data. First, we compare the food consumption of the rural and urban residents as a background, while it is not feasible to conduct statistical tests for the food consumption of rural and urban residents due to the lack of access to the micro household data of the National Household Income and Expenditure Survey in our descriptive part. Thus, we can only draw conclusions with a direct descriptive comparison of rural and urban residents' food consumption with the aggregated data. Second, we concentrate on the analysis of the food purchased externally and do not consider such products which may be unregistered in rural areas due to their own production and consumption. In fact, many rural families in China consume self-produced food. This study does not consider the opportunity costs of obtaining self-produced food due to the lack of relative information. Third, this study only analyzed the main types of food and may not cover all food types, such as soy sauce, vinegar, salt, and many other condiments. The consumption of these items is also necessary for the diet of rural Chinese residents, which may affect the results to some degree. Future studies should consider whole foods including self-produced food, pre-made food, and restaurant food or delivery food, and explore the heterogenous effect of aging on household food consumption between rural and urban residents if needed data are available.

Data availability statement

The datasets presented in this article are not readily available because this is internal agency data. Requests to access the datasets should be directed to the Fixed Observation Point Management Office of the Research Center for Rural Economy under the Ministry of Agriculture and Rural Affairs of China.

Author contributions

MG, BW, and WJ involved in conceptualization. JWang and JL conducted the formal analysis and involved in writing the original draft preparation and methodology. JWang involved in software and performed data curation. MG, JWang, JWei, and WJ involved in the writing, reviewing, and editing. JWang and JWei performed the visualization. JWei and JL involved in language. MG performed the supervision and project administration. MG, JWang, and JL involved in funding acquisition. All authors read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.933343/full#supplementary-material

References

1. Shekar M, Kakietek J, Eberwein JD, Walters D. *An investment framework for nutrition: reaching the global targets for stunting, anemia, breastfeeding, and wasting*. Washington, DC: World Bank (2017).

2. Yang W, Jia H, Wang C, Wang H, Sun C. Spatial heterogeneity of household food consumption and nutritional characteristics of grassland transects in inner mongolia, china. *Front Nutr.* (2022) 9:270. doi: 10.3389/FNUT.2022.810485/BIBTEX

3. Sheng Y, Song L. Agricultural production and food consumption in China: A long-term projection. *China Econ Rev.* (2019) 53:15–29. doi: 10.1016/J.CHIECO.2018.08.006

4. Yu Y, Feng K, Hubacek K, Sun L. Global implications of china's future food consumption. *J Ind Ecol.* (2016) 20:593–602. doi: 10.1111/JIEC.12392

5. Yearbook. *China statistical yearbook: the national bureau of statistics of the people's republic of china*. Sunnyvale, CA: Yearbook (2021).

6. Zou B, Mishra AK, Luo B. Aging population, farm succession, and farmland usage: Evidence from rural China. *Land Use Policy.* (2018) 77:437–45. doi: 10.1016/J.LANDUSEPOL.2018.06.001

7. Zhou Z-Y, Liu H, Cao L. *Food consumption in China: The revolution continues*. Cheltenham: Edward Elgar (2014).

8. Feng Q, Yeung WJJ, Wang Z, Zeng Y. Age of retirement and human capital in an aging china, 2015–2050. *Eur J Populat.* (2019) 35:29–62. doi: 10.1007/S10680-018-9467-3/FIGURES/8

9. Cockx L, Colen L, de Weerdt J. From corn to popcorn? Urbanization and dietary change: Evidence from rural-urban migrants in Tanzania. *World Dev.* (2018) 110:140–59. doi: 10.1016/J.WORLDDEV.2018.04.018

10. Jones AD. On-Farm crop species richness is associated with household diet diversity and quality in subsistence- and market-oriented farming households in malawi. *J Nutr.* (2017) 147:86–96. doi: 10.3945/JN.116.235879

11. Burggraf C, Kuhn L, Q ran Zhao, Teuber R, Glauben T. Economic growth and nutrition transition: an empirical analysis comparing demand elasticities for foods in China and Russia. *J Integr Agric.* (2015) 14:1008–22. doi: 10.1016/S2095-3119(14)60985-0

12. Feyisa MN. The effect of productive safety net programme on household food consumption and dietary diversity in ethiopia. *Front Sustain Food Syst.* (2022) 6:58. doi: 10.3389/FSUFS.2022.714001/BIBTEX

13. Zheng Z, Henneberry SR. Household food demand by income category: evidence from household survey data in an urban chinese province. *Agribusiness.* (2011) 27:99–113. doi: 10.1002/AGR.20243

14. Bai J, Wahl TI, Lohmar BT, Huang J. Food away from home in Beijing: Effects of wealth, time and "free" meals. *China Econ Rev.* (2010) 21:432–41. doi: 10.1016/J.CHIECO.2010.04.003

15. de Oliveira DCRS, de Moura Souza A, Levy RB, Sichieri R, Verly-Jr E. Comparison between household food purchase and individual food consumption in Brazil. *Public Health Nutr.* (2019) 22:841–7. doi: 10.1017/S1368980018002987

16. Hardcastle SJ, Thøgersen-Ntoumani C, Chatzisarantis NLD. Food choice and nutrition: A social psychological perspective. *Nutrients.* (2015) 7:8712–5. doi: 10.3390/NU7105424

17. Morley JE. Decreased food intake with aging. *J Gerontol Ser A.* (2001) 56:81–8. doi: 10.1093/GERONA/56.SUPPL_2.81

18. Ahmed T, Haboubi N. Assessment and management of nutrition in older people and its importance to health. *Clin Interv Aging.* (2010) 5:207. doi: 10.2147/CIA.S9664

19. Seale JL, Bai J, Wahl TI, Lohmar BT. Household engel curve analysis for food, beijing, china. *China Agric Econ Rev.* (2012) 4:427–39. doi: 10.1108/17561371211284795/FULL/XML

20. Min S, J fei Bai, Seale J, Wahl T. Demographics, societal aging, and meat consumption in China. *J Integr Agric.* (2015) 14:995–1007. doi: 10.1016/S2095-3119(14)60984-9

21. Szakos D, Ózsvári L, Kasza G. Perception of older adults about health-related functionality of foods compared with other age groups. *Sustainability.* (2020) 12:2748. doi: 10.3390/SU12072748

22. Kramer LL, Blok M, van Velsen L, Mulder BC, de Vet E. Supporting eating behaviour of community-dwelling older adults: Co-design of an embodied conversational agent. *Design Health.* (2021) 5:120–39. doi: 10.1080/24735132.2021.1885592

23. Nieuwenhuizen WF, Weenen H, Rigby P, Hetherington MM. Older adults and patients in need of nutritional support: Review of current treatment options and factors influencing nutritional intake. *Clin Nutr.* (2010) 29:160–9. doi: 10.1016/J.CLNU.2009.09.003

24. Laureati M, Collier ES, Normann A, Harris KL, Oberrauter L-M, Bergman P. Making more sustainable food choices one meal at a time: Psychological and practical aspects of meat reduction and substitution. *Foods.* (2022) 11:1182. doi: 10.3390/FOODS11091182

25. Vlassoff C. Gender differences in determinants and consequences of health and illness. *J Health Popul Nutr.* (2007) 25:47–61.

26. Lobos G, Grunert KG, Bustamante M, Schnettler B. With health and good food, great Life! Gender differences and happiness in chilean rural older adults. *Soc Indic Res.* (2016) 127:865–85. doi: 10.1007/S11205-015-0971-0/TABLES/4

27. Roudsari AH, Vedadhir A, Amiri P, Kalantari N, Omidvar N, Eini-Zinab H, et al. Psycho-Socio-Cultural determinants of food choice: A qualitative study on adults in social and cultural context of iran. *Iran J Psychiatry.* (2017) 12:241–50.

28. Denton M, Walters V. Gender differences in structural and behavioral determinants of health: an analysis of the social production of health. *Soc Sci Med.* (1999) 48:1221–35. doi: 10.1016/S0277-9536(98)00421-3

29. Herne S. Research on food choice and nutritional status in elderly people: A review. *Br Food J.* (1995) 97:12–29. doi: 10.1108/00070709510100136/FULL/PDF

30. Jin S, Zhang B, Wu B, Han D, Hu Y, Ren C, et al. Decoupling livestock and crop production at the household level in China. *Nat Sustain.* (2020) 4:48–55. doi: 10.1038/s41893-020-00596-0

31. Xu C, Holly Wang H, Shi Q. Farmers' income and production responses to rural taxation reform in three regions in china. *J Agric Econ.* (2012) 63:291–309. doi: 10.1111/J.1477-9552.2012.00338.X

32. He G, Wang S. Do college graduates serving as village officials help rural China? *Am Econ J Appl Econ.* (2017) 9:186–215. doi: 10.1257/APP.20160079

33. Zhang R, Wei T, Glomsrød S, Shi Q. Bioenergy consumption in rural China: Evidence from a survey in three provinces. *Energy Policy.* (2014) 75:136–45. doi: 10.1016/J.ENPOL.2014.08.036

34. Pitt MM, Rosenzweig MR. Health and nutrient consumption across and within farm households. *Rev Econ Stat.* (1985) 67:212. doi: 10.2307/1924720

35. Min S, Ling-ling HOU, Hermann W, Ji-kun HUANG, Y ying MU. The impact of migration on the food consumption and nutrition of left-behind family members: Evidence from a minority mountainous region of southwestern China. *J Integr Agric.* (2019) 18:1780–92. doi: 10.1016/S2095-3119(19)62588-8

36. Bouis HE. The effect of income on demand for food in poor countries: Are our food consumption databases giving us reliable estimates? *J Dev Econ.* (1994) 44:199–226. doi: 10.1016/0304-3878(94)00012-3

37. Walton K, Horton NJ, Rifas-Shiman SL, Field AE, Austin SB, Haycraft E, et al. Exploring the role of family functioning in the association between frequency of family dinners and dietary intake among adolescents and young adults. *JAMA Netw Open.* (2018) 1:e185217–185217. doi: 10.1001/JAMANETWORKOPEN.2018.5217

38. Lechene V, Pendakur K, Wolf A. Ordinary least squares estimation of the intrahousehold distribution of expenditure. *J Polit Econ.* (2022) 130:681–731. doi: 10.1086/717892/SUPPL_FILE/20200183DATA.ZIP

39. Huang Y, Tian X. Food accessibility, diversity of agricultural production and dietary pattern in rural China. *Food Policy.* (2019) 84:92–102. doi: 10.1016/J.FOODPOL.2019.03.002

40. He Y, Yang X, Xia J, Zhao L, Yang Y. Consumption of meat and dairy products in China: a review. *Proc Nutr Soc.* (2016) 75:385–91. doi: 10.1017/S0029665116000641

41. Yu X. Engel curve, farmer welfare and food consumption in 40 years of rural China. *China Agric Econ Rev.* (2018) 10:65–77. doi: 10.1108/CAER-10-2017-0184/FULL/XML

42. Larson RW. The solitary side of life: An examination of the time people spend alone from childhood to old age. *Dev Rev.* (1990) 10:155–83. doi: 10.1016/0273-2297(90)90008-R

43. Ledikwe JH, Smiciklas-Wright H, Mitchell DC, Miller CK, Jensen GL. Dietary patterns of rural older adults are associated with weight and nutritional status. *J Am Geriatr Soc.* (2004) 52:589–95. doi: 10.1111/J.1532-5415.2004.52167.X

44. Yu X, Abler D. Matching food with mouths: A statistical explanation to the abnormal decline of per capita food consumption in rural China. *Food Policy.* (2016) 63:36–43. doi: 10.1016/J.FOODPOL.2016.06.009

45. Howarth NC, Huang TTK, Roberts SB, Lin BH, McCrory MA. Eating patterns and dietary composition in relation to BMI in younger and older adults. *Int J Obes.* (2006) 31:675–84. doi: 10.1038/sj.ijo.0803456

46. Browning CJ, Qiu Z, Yang H, Zhang T, Thomas SA. Food, eating, and happy aging: The perceptions of older Chinese people. *Front Public Health.* (2019) 7:73. doi: 10.3389/FPUBH.2019.00073/BIBTEX

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Crocin: Functional characteristics, extraction, food applications and efficacy against brain related disorders

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Crocin is a bioactive compound that naturally occurs in some medicinal plants, especially saffron and gardenia fruit. Different conventional and novel methods are used for its extraction. Due to some control conditions, recent methods such as ultrasonic extraction, supercritical fluid extraction, enzyme-associated extraction, microwave extraction, and pulsed electric field extraction are widely used because these methods give more yield and efficiency. Crocin is incorporated into different food products to make functional foods. However, it can also aid in the stability of food products. Due to its ability to protect against brain diseases, the demand for crocin has been rising in the pharmaceutical industry. It also contain antioxidant, anti-inflammatory, anticancer and antidepressant qualities. This review aims to describe crocin and its role in developing functional food, extraction, and bioavailability in various brain-related diseases. The results of the literature strongly support the importance of crocin against various diseases and its use in making different functional foods.

KEYWORDS

crocin, extraction techniques, food applications, medicinal, brain disorders

Introduction

Crocins, a series of polyene dicarboxylic acid, mono and di-glucosyl esters of crocetin, are the major colors causing compounds of saffron and gardenia. In China, the contents of gardenia fruits are used as herbal remedies and natural colors (1). Other plants, such as *Buddleja spp*., also generate crocins, but because of their low concentration, they are not commercially utilized (2). Crocin is a chemical diester composed of the dicarboxylic acid crocetin and disaccharide gentiobiose (3). Crocins are crocetin glycosyl esters generated *via* the esterification of crocetin with various glycosides, including geometric isomers (3). The activity of glucosyltransferases causes the transfer of crocetin molecules, which add varying amounts of glycosidic to yield crocins, a primary component of saffron that confers water solubility (4). Crocins are responsible for many of this valuable plant's pharmacologic effects (5). Crocins, unlike other carotenoids, have 20 carbons and several glycosides. Several earlier studies demonstrated that crocins, particularly alpha crocin, had radical quenching, antioxidant, and anti-inflammatory properties (6).

Crocin (mono- or di-glycosyl polyene esters) is a key bioactive component in saffron that dissolves easily in water and produces a distinctive red color, making saffron a natural coloring compound (7). Trans-crocetin di-(-D-gentiobiosyl) ester, crocin1 is the most prevalent crocin, with a golden-yellow tint. Crocin has the highest recorded absorbance at 440 nm (8). Because of its limited stability, crocins lose its functionality when exposed to light, heat, and acidic nature (9). Also have low immersion and bioavailability, as it is un-absorbed if taken orally till hydrolyzed to produce deglycosylated trans-crocetin in the intestinal tract by enzymatic conditioning in the intestinal epithelial cells and by the fecal bacteria (9).

Crocin has several pharmacological actions, including anti-inflammatory and cancer cell growth inhibitor properties (10–12). Under various experimental settings, crocin has also been shown to protect against oxidative damage to brain vasculature, renal tissues, the heart, and the retina (3). In addition to their anti-hypertensive, anti-platelet aggregation, nephron-protective effects, anti-depressant, and anti-atherosclerotic, these phytoconstituents are radical scavengers, particularly against superoxide anions (13). Many people are afflicted with neurodegenerative disorders such as epilepsy, Parkinson's, and Alzheimer's, with increasing age being the primary risk factor (14). The naturally occurring carotenoid molecule, crocin, has been shown to offer therapeutic potential in treating neurological disorders (15). Crocin also increases dopamine in the brain during Parkinson's disease. As a result, this chemical has been demonstrated to be a promising treatment option for neurodegenerative diseases (16). According to randomized, double-blind, placebo-controlled experiments, crocin medicinal levels do not harm the body. In a double blind randomized clinical trial it was found that crocin (20 mg/day) is safe in healthy volunteers, with no notable changes in hematological, hormonal, biochemical, or urine markers (17). This review aims to describe the role of crocin in developing functional food, extraction and critically evaluate prior and current findings on the biological/pharmacological actions of crocin against various brain related diseases.

Biochemical structure of crocin family

The crocins are a class of hydrophilic carotenoids that are either mono- or di-glycosyl polyene esters of crocetin in which D-gentiobiose and/or D-glucose appear as carbohydrate residues (18). A carotenoid with a 20-carbon dicarboxylic structure. Saffron contains a variety of carotenoid chemicals, containing trace levels of zeaxanthin, alpha and beta carotene, and lycopene (19).

Six different forms of the crocin family's glycosyl esters have been found in saffron. Trans-crocins 3 and 4 are the most prevalent of the crocin analogs, which include crocins 1–4 and are virtually glycosides of trans-crocetin in saffron. These crocins range in concentration in Spanish saffron between 0.01 and 9.44 percent and 0.46 and 12.12 percent, respectively, whereas cis-crocetin and its glycosides are minor constituents (20). Except for crocin-1, all crocin derivatives are said to exist as pairs of cis-trans isomers. Trans-crocins undergo photoisomerization events and change to cis-crocins, according to a research by (21); this process is reliant on the agro-ecological circumstances in the region of the plant's origin.

Due to its high water solubility, crocin, also known as alltrans crocetin di-b-D-gentiobiosyl ester, has the best coloring capacity when compared to the other carotenoides of saffron. Crocin, which is also soluble in methanol and ethanol, is considered as the preferred water-soluble food additive because of its ability to quench free radicals and possess tumor-fighting characteristics. Structure of crocetin and its glycosides are presented in (Figure 1).

Different plant sources of crocin

Crocin is the pigment that gives saffron its color (22). It is also taken from the fruits of the gardenia (23). It occurs as a red powder as a solid, yet it gives a yellow color to dishes (24). crocin as a spice produced by the s *Crocus Sativus* L. is produced primarily in Western Asia, with Iran being the world's largest producer, but it is also economically significant in huge sections of Mediterranean Europe (25). The bitter flavor of a saffron spice is attributed to the monoterpene glycoside picrocrocin, whereas the scent is attributed to the aglycone safranal (26). The closed flowers of saffron are hand-picked in the early morning to ensure greater resistance to the degenerative processes of the floral organs and obtain a spice with high qualitative traits (25). A mechanical system can also do harvesting with two primary parts: the first detaches the corolla from the stem, and the second gathers the removed flower using a vacuum collector (25). It takes 370–470 h to make 1 kg of dried saffron through manual plucking (27). Using high-performance liquid chromatography (HPLC), the principal features of the saffron extract related to crocins and safranal concentration were identified (28).

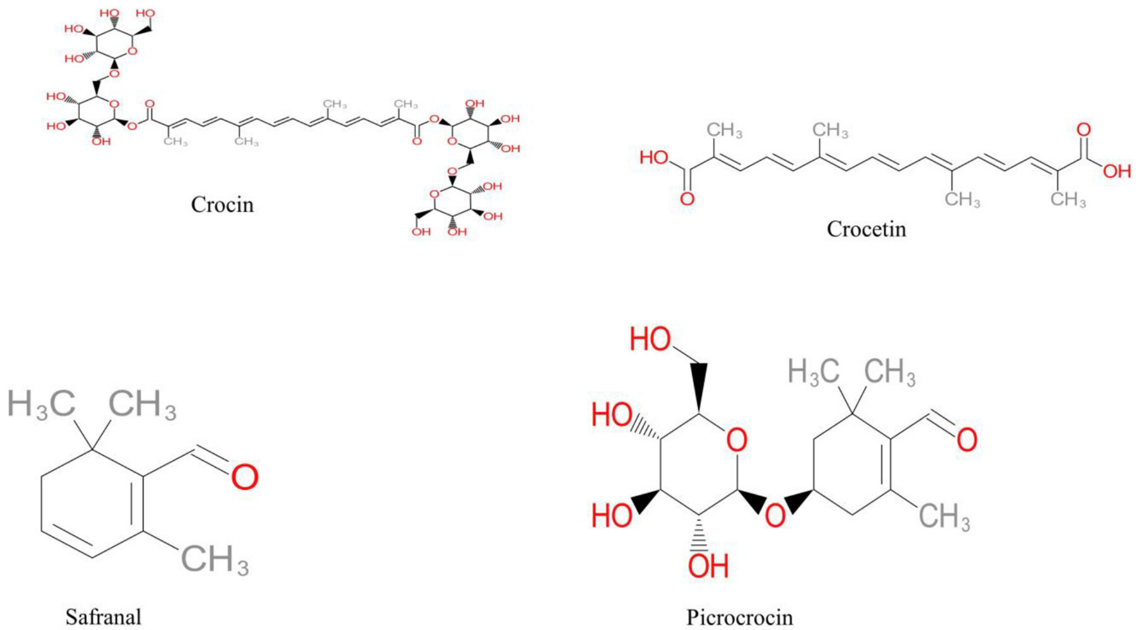

FIGURE 1
Chemical structure of crocin, dicarboxylic acid (crocetin), monoterpen aldehyde (safranal) and glycoside (picrocrocin).

Gardenia is an evergreen shrub commonly used in landscaping with characteristics like sweet, softly aromatic blossoms (29). It is a well-known fruit for ayurvedic purposes in China due to its chilly and bitter characteristics (30). Therefore, medicinal importance of this shrub includes curing stomach aches and hepatic, as well as treat diuretic, anti-phlogistic, choleretic, laxative, and homeostatic qualities (30). Also, it is used to obtain yellow color since it contains crocin and crocetin, primary plant carotenoid constituents (30).

The derivatives derived from *G. jasminoides* are less poisonous, less allergenic, and more environmentally friendly than saffron (31). A homogenate extraction procedure was used to extract crocin from *G. jasminoides* (32, 33). Another work used ethanol/water cold percolation to extract crocin from *G. jasminoides* without affecting percolation (34). The Microwave-assisted extraction (MAE) method boosted the extraction yield of crocin from *G. jasminoides*' edible yellow pigment by 50% over the usual extraction method (35).

Crocin microbial production has attracted significant interest recently, but its implementation is limited as per the literature studies (36). Carotenoids such as astaxanthin, lycopene, and carotene have been genetically engineered into *Escherichia coli (E. coli)*. As a result, experiments were done to develop *E. coli* cell factories that could produce crocin. *E. coli* has a distinctive genetic make-up, expands quickly under simple culture conditions, and is capable of a variety of well-known large-scale fermentation processes. The YL4 and YL5 strains are the ones that start crocin synthesis. Finally, the researchers were able to establish strains that produced crocetin and crocin-5 by integrating and optimizing the expression of the heterologous genes (37).

Extraction of crocin by conventional method and novel techniques

Extracting bioactive components from saffron necessitates ongoing research into environmentally and economically viable extraction strategies (38, 39). Traditional extraction procedures are time-consuming and necessitate a large amount of solvent (40). As a result, novel extraction strategies for extracting bioactive components from saffron have been devised, reducing extraction time and solvent usage while improving extract extraction yield and quality (38). Several approaches have been developed to extract bioactive components from saffron with maximum extraction and purity efficiency (38). It is confirmed that, compared to traditional procedures, the targeted bioactive components can be extracted more efficiently in terms of solvent volume and extraction time by employing the right extraction method (41–43). According to El Asbahani et al. (44), the extraction method should be chosen based on the desired bioactive component, heat sensitivity, tissue complexity, etc. Conventional extraction processes (soxhlet extraction, maceration, solvent extraction vapor, or hydro-distillation) are generally non-selective, require a high volume of organic solvents, and require longer extraction times in

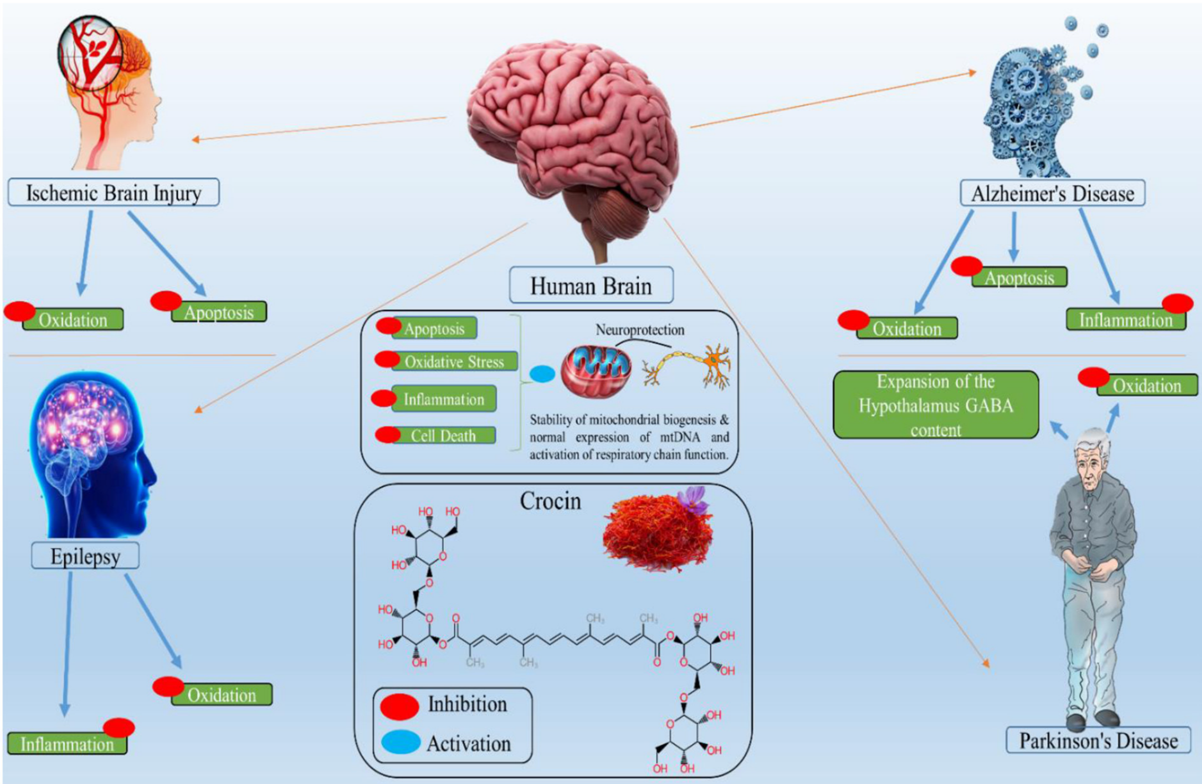

FIGURE 2
Biological activity of crocin in different brain related disorders, where the red circle shows the inhibitory property of crocin and blue circle shows the activation.

certain cases, damage heat-sensitive bioactive chemicals (16, 45). These current extraction processes, known as "green methods," are environmentally friendly, safer, faster, more efficient, and more precise (46). Green approaches include several extraction techniques, including membrane and emulsion liquid ultrasonic extraction, supercritical fluid extraction, enzyme-associated extraction, microwave extraction, and pulsed electric field extraction (41, 47). These approaches can efficiently extract saffron bioactive components. In general, the efficiency of extraction procedures is primarily determined by the selection of appropriate solvents, taking into account solvent-solute affinity and the employment of coextraction techniques (48). To get saffron bioactive components such as crocin, picrocrocin, and safranal, a wide range of solvents, such as water and organic solvents, and their combinations have been utilized (49). In general, fewer polar chemicals (safranal) are extracted with carbon dioxide, whereas initially, polar substances (crocin, crocetin) are extracted with organic solvents (e.g., ethanol) (50). Mohajeri et al. (51) demonstrated the extraction of crocin from saffron using molecularly imprinted polymer methods. Recent research on saffron used MAE to extract several bioactive components (picrocrocin, safranal, and crocin). The components' contents were determined spectrophotometrically at 257, 330, and 440 nm (the peak absorbance values of picrocrocin, safranal and crocin), respectively (52). The extraction and purification techniques depend on obtaining any important elements such as bioactive chemicals (crocins, crocetin, safranal, and picrocrocins) naturally found in plants. An effective bioactive extraction process should fulfill green chemistry standards such as safety, environmental friendliness, low or no contaminants, efficiency, and economics (41, 53). Saffron's key bioactive components—picrocrocin, safranal, and crocin—were extracted using subcritical water. The response surface approach was used to study the effect of extraction time (5–15 min) and temperature (105–125°C) on process efficiency. The crystallization process was used to recover complete crocin from saffron stigmas. The optimal extraction solvent was determined to be 80% ethanol. At different temperatures, the crystallization process was carried out in one and two. As a crystallization media, 80% ethanol was employed. Crocin crystals were obtained with low purity from 1st crystallization as compared to 2nd crystallization produced pure crystals at a low temperature (−5°C) quantified using UV-visible spectrophotometer and HPLC followed by Fluka product and

TABLE 1 Extraction and separation of crocin.

Source	Extraction (conventional/ novel)	Extraction technique	Conditions	Detection/separation	References
Gardenia fruits	Conventional	50% acetone	Time: 31.4–32.2 min Temperature: −40°C	HPLC	(56)
Saffron	Conventional	Methanol–water (50:50, v/v)	Time: 24 h Temperature: 4°C	HPLC	(57)
Saffron	–	–	Time: 05 min Room temperature	HPLC	(58)
Green ovaries gardenia	Conventional	50% (v/v) ethanol	Time: 45 min Room temperature	HPLC	(59)
Gardenia fruits	–	–	Time: 28.6 min Temperature: 70.4°C	HPLC	(60)
Saffron stigma	Conventional	Water	–	Thin layer chromatography	(61)
Saffron	Conventional	Methyl alcohol	–	–	(62)
Saffron	–	–	–	Electronic tongue	(63)
Saffron	Conventional	Ethanol	Time: 4 and 3 h Temperature: 100°C	–	(64)
Saffron	Novel	Microwave-assisted extraction (MAE)	Time: 2–10 min Temperature: 100–103°C	–	(52)
Saffron	Novel	Microextraction	–	–	(65)
Saffron	Novel	Ultrasound	–	–	(66)
Saffron	Novel	Homogenate extraction	Time: 40 S Temperature: 57°C	–	(67)
Saffron	Novel	Ultrasound-assisted extraction (UAE)	–	HPLC	(68)
Crocus sativus L. dry stigmas	Novel	Ultrasound assisted extraction	–	HPLC	(69)
Saffron stigma	Novel	Ultrasound assisted extraction	–	UV-vis spectrophotometer	(70)
Saffron	Novel	Microwave-assisted extraction	–	–	(71)
Saffron	Novel	Supercritical carbon dioxide extraction	–	HPLC	(72)

saffron extraction using methanol (54, 55). The results showed that its purity was ∼13 times greater than the Fluka products. Despite our expectations, the Fluka product was not a pure alpha-crocin sample; its chromatogram revealed five forms of crocins and an unknown impurity (54). The purity of total crystalline crocin was >97% (54). Crocin can only be split into seven fractions using this approach. The approach also necessitates time-consuming multiple treatments before pure crocin can be extracted. Table 1 shows the conventional and novel extraction methods of crocin.

Food applications of crocin

Saffron is a major source of various bioactive compounds, including crocins, picrocrocin, and safranal (49). In various food products, such as bakeries and beverages, the stigma of saffron is widely used as a coloring agent and an aroma (73). Studies also show that different parts of the saffron plant are used in the development of functional food products (74). The development of functional cookies enriched with the stigma of saffron showed distinct attributes like good sensory scores, increased shelf life, and high antioxidant activities (75). Different beverages enriched with saffron petals demonstrate increased antioxidant properties after fermentation (76). Various products are available in the market encapsulated with bioactive compounds commonly found in the stigma and petals of saffron (41). The enrichment of saffron bioactive ingredients is done on a large scale. The saffron-enriched pasta products exhibit a low glycemic index due to resistant starch digestibility (77). It was also observed that the crocin-encapsulated tablet decreased the glycemic index due to reduce amylase activity in healthy people (78). Saffron carotenoids and crocin are used as coloring agents. Various food dishes are prepared with crocin, like the low kebab in Iran, pulao in India, and paella rice in Spain (Table 2).

Dairy products

The bioactive compounds of saffron are commonly used to develop various functional food products, including dairy products (88). Moreover, in dairy products, various types of cheese are developed that are enriched with saffron (89). Various cheeses, including Luneberg cheese, Pecorino allo Zafferano and Piacentinu Ennese cheese, are obtained from different animal milk sources like Austrian cow's milk, Italian sheep's milk, and Sicily sheep's milk. Enriching saffron ingredients like crocin in sheep's milk influenced physicochemical properties (90). It was evident from studies that cheese enriched with saffron showed more distinct properties than others (91). This type of cheese is yellowish in color having good elasticity and microbiologically more stable (134). A group of researchers examined the effect of saffron-rich cheese in different aspects, including physicochemical properties, sensory attributes, color, and antioxidant activities. No significant change occurred in all properties, but the saffron-enriched product's antioxidant activity and antimicrobial capacity increased (91).

Dessert products

In dessert products, two types of dessert products, cheesecake incorporated with saffron and orange jam; the other white chocolate soup incorporated with saffron and yogurt, were evaluated in various physicochemical properties (92). A standardized level of crocin was incorporated in both types of desserts. It was concluded that the dessert incorporated with crocin had increased consumer acceptability and precise control dosage compared to dessert incorporated with saffron stigma (135). Several factors like easily water-soluble, undesirable fibers, and the small size of powder saffron do not affect the properties of the desired product. The saffron extract is considered more valuable due to its uniform color intensity and no need for preheating treatments (9).

TABLE 2 Food applications of crocin.

Plant source	Food application	Purpose to make functional food	References
–	W/O micro-emulsions	Encapsulation	(79)
Saffron	–	Nano encapsulation	(80)
Saffron	Crocin emulsion	–	(81)
Saffron	–	Nano encapsulated crocin	(82)
Saffron	Pasta	Saffron extract (crocin) seems to effect starch digestibility	(83)
–	Acidic beverages	Results show the improvement by addition of soybean soluble polysaccharide (SSPS) and avenin on the compound properties and stability of crocin in different conditions [under acidic (pH 2.5) and neutral (pH 7.0)].	(84)
Saffron	Drink	It may be given a sweet taste and aroma to the drink (wine, other alcoholic and non-alcoholic beverages)	(85)
Gardenia fruit pomace (GFP)	Steamed bread	GFP enrichment of steamed bread could retain most of the crocins and slow starch digestion that improves the appearance and nutritional quality of steamed bread.	(86)
Stigma (*Crocus sativus*)	Rye bread	The results showed a most consumer-acceptable rye bread (RB) containing saffron (S) powder (RB + S) was designed to verify its anti-diabetic properties.	(87)
Saffron	Wheat flour pasta	Saffron improved the antioxidant activity and crocin content of the pasta.	(77)

Cereal products

The applications of saffron in cereal products are very effective in reducing disease risk and improving the health status of the modern-age population (93). The incorporation of saffron bioactive compounds, especially crocin, in cereal products is examined in several aspects like physicochemical properties, color, texture, and sensory attributes (94). The pasta incorporated with saffron showed distinct variations in different properties, including aroma, taste, color, gumminess, hardness, chewiness, and overall acceptability (95). It was evident from the DPPH and ABTS assays that saffron-incorporated pasta showed high antioxidant properties (95). Several studies showed that the water uptake during the cooking process of pasta incorporated with a high amount of saffron is reduced due to non-soluble compounds found in saffron that are responsible for inhibiting the diffusion of water in the gluten matrix (96).

Beverage industry

Saffron extract is widely used in the beverage industry to prepare alcoholic and non-alcoholic beverages, herbal teas, vermouth, and several bitter drinks (97). In beverages, the bitterness due to saffron extract is a limited aspect of consumers' acceptance (98). It was observed that the phenolic content in the herbal tea blend improves the bioaccessibility of crocin by reducing the crocin oxidation during the digestion process. The previous studies showed the bioavailability and bioaccessibility of saffron carotenoids in beverage industries (99).

Effect of crocin on brain-related diseases

Crocin is a natural neuroprotective molecule with anti-depressant properties and potential use in treating neuropsychological problems (100). Crocin has been discovered to reduce beta-amyloid aggregation, a key step in Alzheimer's disease pathogenesis. Crocin helps those with chronic obstructive pulmonary disease and lipopolysaccharide who are depressed. Crocin can also act as an anti-inflammatory agent (101). Figure 2 presents the biological activity of crocin in different brain-related disorders. The pharmacological potential of crocin in brain-related diseases is shown in Table 3.

Alzheimer's

Alzheimer's is a neurodegenerative disease that causes mental capacity development and disrupts neurocognitive functions (109). Neurodegeneration, neuronal loss, and the formation of neurofibrillary tangles and Ab plaques are all signs and symptoms of this neuropathological disorder (109). Alzheimer's disease is the major cause of dementia in people over 60. Alzheimer's disease affects between 50 and 75% of patients with dementia (109). There is a lack of a logical chronological order of events in Alzheimer's disease and acceptable and effective treatment (110). The interaction of Ab protein oligomers with glial cells and neurons causes a variety of pathological and physiological abnormalities, including mitochondrial dysfunction, pro-inflammatory cascade stimulation, increased tau phosphorylation and oxidative stress, calcium metabolism deregulation, increased glycogen synthase kinase (GSK)-3b activity, cell death stimulation, and neuronal apoptosis (111, 112).

Alzheimer's disease is a difficult illness to prevent and treat because of its complex pathophysiology (113). Herbal compounds have been proposed as potential anti-Alzheimer's agents (114). Crocin, the primary component extracted from *Crocus sativus* L, has various pharmacological effects, including anti-apoptotic activity. Crocin, the primary component of *Crocus sativus* L. extract, is a yellow carotenoid with anti-inflammatory, anti-depressant, and memory-improving qualities, as well as various pharmacological activities, including anti-apoptotic capabilities (115).

Crocin's neuroprotective actions boost memory by scavenging free radicals, reducing the synthesis of peroxidized membrane lipids, and reestablishing SOD activity, reducing ROS and AGEs while lowering MDA levels and increasing GPx activity. Crocin's antioxidant effect effectively protects cerebrocortical and hippocampal neurons from ischemia, improving spatial cognitive abilities. Crocin modulated Mitogen-Activated Protein Kinase, which suppressed A concentration (MAPK) and tau phosphorylation, reducing acrolein-induced oxidative pressure. Acrolein has been linked to the development of Alzheimer's disease. In rats, acrolein at 3 mg/kg/day p.o. lowers Glutathione (GSH) levels, increases MDA, A, and Pt in the brain, and activates MAPK signaling pathways (16).

Parkinson's disease

Parkinson's disease (PD) is the most prevalent neurological disorder. It is a progressive neurological disease that primarily affects the elderly (116). Anxiety, depression, sleep problems, and cognitive modifiability are common neuropsychiatric diseases in people with Parkinson's disease. For people with Parkinson's disease, these disturbances often create greater difficulty and distress than the disease's motor symptoms. Depression is the most frequent neuropsychiatric symptom in Parkinson's disease, with up to 50% of PD patients suffering from this psychiatric illness. The loss of nigrostriatal dopamine (DA) neurons is a characteristic symptom of Parkinson's disease (101). Development of filamentous, cytoplasmic inclusions, primary aggregations of synuclein as Lewy bodies (LB) or neurites is a pathological characteristic of PD. Fibrillization and Synuclein phosphorylation lead to the development of LB and neuron death. In Parkinson's patients, synuclein aggregates have been observed in the dorsal motor nucleus (DMN), vagus nerve, spinal sympathetic epicardium nerves, and preganglionic neurons. In 60% of PD patients with bladder hypersensitivity, urinary tract abnormalities are identified, resulting in voiding urgency, incontinence, and frequency (117).

TABLE 3 Pharmacological potential of crocin in brain-related disease.

Food/food part	*In vivo/in vitro*	Disease (brain)	Improvement	References
Crocus sativus L	–	Chronic stress	Treatment with saffron extract or crocin blocked the ability of chronic stress to impair spatial learning and memory retention	(20)
Stigma	–	Alzheimer disorder, brain neurodegenerative disorder	It has universal acceptability as a phytotherapeutic drugs	(24)
Saffron	*In vivo*	Withdrawal syndrome, craving, and cognitive function	Results showed that crocin supplementation for 12 weeks to patients under MMT programs had beneficial effects on craving and withdrawal symptoms score, but did not affect the cognitive function parameters.	(102)
Saffron	*Iv vivo*	Chronic cerebral hypo perfusion	The study suggests that saffron extract and crocin improve spatial cognitive abilities following chronic cerebral hypo perfusion.	(103)
Dry stigma of the plant *Crocus sativus L.*	–	Alzheimer's disease (AD)	The results showed that crocin potential to improve learning and memory as well as protect brain cells	(104)
Saffron	*In vivo*	Brain aging (Cognitive decline and memory deficits)	This study suggested that saffron-treated mice exhibited significant improvement in learning and memory	(105)
Saffron	*In vivo*	Mild traumatic brain injury	Saffron extract and crocin provide a neuroprotective effect in a mouse model of rmTBI by decreasing oxidative stress and inflammatory responses.	(106)
Fruits of gardenia and stigmas of saffron	*In vivo*	CNS homeostasis	The finding showed that crocin and crocetin provide neuroprotection by reducing the production of various neurotoxic molecules from activated microglia.	(107)
Saffron	*In vivo*	–	The biochemical changes support the neuroprotective potential of saffron under toxicity.	
–	–	Alzheimer's disease	The result suggested that crocin may have beneficial effects in the treatment of neurodegenerative disorders (Alzheimer's disease).	(108)

Furthermore, it has been demonstrated that crocin treatment can inhibit AChE activation from increasing. As a result of these qualities, we chose to test crocin's neuroprotective effects against dopaminergic neuron damage and PD consequences in a model (mouse model) of this disease through 1-methyl4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Crocin also lowers depressive-like symptoms such as anxiety in adult male rats exposed to teen stress and dendritic remodeling in the PFC (prefrontal cortex). Crocin has been demonstrated as effective for disease in several investigations. Crocin also enhanced aversive memory in a Parkinson's patient model based on 6-hydroxydopamine. These findings show that crocin could be a novel contender for Parkinson's disease and depression treatment (101).

Crocin has also been shown to lower the amount of -synuclein in rats with rotenone-induced Parkinson's disease. As a result, the protective impact of crocin (10, 20, 40 mg/kg, 28 days, i.p.) on oxidative stress, malathion-induced Parkinson's disease, and inflammation in the rat striatum were examined (118).

In another animal model of disease caused due to MPTP, crocin treatment was found to reduce motor deficits and preserve dopaminergic neurons and by blocking the opening of the mitochondrial permeability transition pore (mPTP) protect against mitochondrial dysfunction (119).

Ischemic brain injury

Hypoxic-ischemic brain damage could lead to morbidity and mortality among all age groups. One of the most common causes of infant brain damage is hypoxic-ischemic (HI) injury. In the United States, 1–4 neonatal HI brain injury occurrences occur per 1,000 live births, accounting for around one-fourth of all neonatal deaths globally. Intrauterine hypoxia related to circulation issues, such as placental abruption, placental artery clotting, and inflammatory processes, is the most common cause of neonatal hypoxic brain injury (120). Using these models, researchers have discovered numerous distinct characteristics of neonatal HI brain injury, which may be related to the nervous system's immaturity. Around birth, antioxidant enzymes (e.g., copper-zinc superoxide dismutase and glutathione peroxidase) have a restricted action in the young brain. As a result, oxidative damage induced by HI injury is more likely in the infant's brain (121).

Crocin is an active ingredient isolated from saffron with anti-inflammatory, antioxidant, and neuroprotective effects. According to a previous study, crocin pretreatment reduced cerebral edema and enhanced functional outcomes in a mouse model of traumatic brain injury. Crocin was also reported to reduce brain infarct volume in the rat ischemia-reperfusion paradigm. It is unknown whether crocin has a neuroprotective effect on HIE (122).

Crocin's therapeutic impact in reducing blood brain barrier (BBB) disruption was investigated. Twenty-four-month-old rats were administered either vehicle (controls) or crocin (10, 20, 40, or 60 mg/kg) every other day for 2 months before ischemia induction. In the presence of cerebral ischemia, Crocin preserved BBB function. In addition, the crocin-treated group had increased NADPH oxidase. The antioxidant ability of crocin was shown in these experiments to help minimize the damage induced by ischemia (123).

Epilepsy

Epilepsy, a neurological disorder marked by recurring seizures, is frequently linked to earlier nervous system abnormalities. Epilepsy is caused by a disruption in the regulation of inflammatory cell activation and resolution in injured neuronal tissue. However, this imbalanced inflammatory regulation that contributes to epilepsy is still unknown (124). Epileptic convulsions are due to disruptions in the physiology of the brain. Abnormalities in the membrane properties of neurons, decreased inhibition of neurotransmission (by gamma-aminobutyric acid, GABA), changes in the ionic microenvironment surrounding the neuron, or increased excitatory neurotransmission (by the acidic amino acid glutamate) are all causes of epileptic seizures. In all ionotropic glutamate receptors, the intake of sodium and the outflow of potassium ions by the channels can depolarize the membrane by forming the action potential. In the resting state, Mg^{++} ions block the Ca^{++} channel in NMDA receptors, depolarizing the local membrane; channels change to permeable for calcium ions with the shifts of Mg^{++}. In high neuronal activation (e.g., status epilepticus), Ca^{++} inflow can cause cell depolarization to play a part in Ca^{++}-mediated neuronal damage, leading to cell death (125).

Because epilepsy involves complicated neural pathogenic pathways, multi-targeted pharmacological treatments have been proposed for its complete care. It is been studied extensively in animal models of neurological disorders, including depression, epilepsy, anxiety, and memory. Crocin's efficacy in neurological diseases characterized by aberrant central excitatory and inhibitory nature shows that it interacts with various neuronal pathophysiological pathways (126).

Crocin has an unsettling effect on the cell reinforcement framework, resulting in increased ROS production and subsequent ROS-induced mitochondrial malfunction, frequently found following seizures and throughout epileptogenesis. Its antioxidant properties also aid crocin's antiepileptic properties. Antiepileptic medicines improve GABA-mediated inhibition and raise GABA levels in the brain (16). Crocin infusions in penicillin-activated epileptiform action in rats resulted in antiepileptic effects. Crocin's antiepileptic effect is due to its ability to increase the tone of inhibitory neurotransmitters by increasing the working of GABA (A)-benzodiazepine. Crocin stimulates glutamic acid decarboxylase, an important enzyme for GABA synthesis, greatly increasing the hypothalamic GABA concentration in rats (127).

Conclusions

The present study is deliberate to measure the effects of crocin extract in functional foods and its pharmacological properties against various disorders. Crocin is a biologically active substance in the stigma of saffron and fruit of guardian. These bioactive substance can be extracted utilizing conventional (solvent extraction, soxhlet extraction, vapor or hydro distillation, and maceration), and novel techniques (supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed electric field extraction, emulsion liquid membrane extraction and enzyme-associated extraction). In various food products, such as bakeries and beverages, the stigma of saffron is widely used as a coloring agent and an aroma. The antioxidant profile of crocin, may inhibit the oxidation process in different foods. The development of baking products, beverages, dairy products, dessert and cereal products are enriched with the stigma of saffron showed distinct attributes (sensory scores, good shelf life, antioxidant activity). These products having saffron in them have very effective in reducing disease risk and improving the health status of the population. Pharmacologically, crocin may be helpful in different brain-related disorders, including Alzheimer's, Parkinson's, Ischemic brain injury, and Epilepsy.

Author contributions

AAl, SK, WK, and MM: conceptualization. AAl, SK, WK, ZM, AAz, and MA: writing-original draft preparation. RA, MT, LY, HQ, SR, HS, and MM: writing-review and editing. LY, HQ, and MM: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Pan Y, Zhao X, Wang Y, Tan J, Chen D-X. Metabolomics integrated with transcriptomics reveals the distribution of iridoid and crocin metabolic flux in Gardenia jasminoides Ellis. *PLoS ONE.* (2021) 16:e0256802. doi: 10.1371/journal.pone.0256802

2. Martí M, Diretto G, Aragonés V, Frusciante S, Ahrazem O, Gómez-Gómez L, et al. Efficient production of saffron crocins and picrocrocin in Nicotiana benthamiana using a virus-driven system. *Metab Eng.* (2020) 61:238–50. doi: 10.1016/j.ymben.2020.06.009

3. Cerdá-Bernad D, Valero-Cases E, Pastor J-J, Frutos MJ. Saffron bioactives crocin, crocetin and safranal: Effect on oxidative stress and mechanisms of action. *Crit Rev Food Sci Nutr.* (2022) 62:3232–49. doi: 10.1080/10408398.2020.1864279

4. Moratalla-López N, Bagur MJ, Lorenzo C, Martínez-Navarro M, Salinas MR, Alonso GL. Bioactivity and bioavailability of the major metabolites of crocus sativus L. *Flower Molecules.* (2019) 24:2827. doi: 10.3390/molecules24 152827

5. Dhiman N, Kharkwal H. *Biosynthesis and Derivatization of the Major Phytoconstituents of Saffron*. Saffron: Elsevier (2020). p. 83–92.

6. Abedimanesh N, Motlagh B, Abedimanesh S, Bathaie SZ, Separham A, Ostadrahimi A. Effects of crocin and saffron aqueous extract on gene expression of SIRT1, AMPK, LOX1, NF-κB, and MCP-1 in patients with coronary artery disease: a randomized placebo-controlled clinical trial. *Phytother Res.* (2020) 34:1114–22. doi: 10.1002/ptr.6580

7. Cid-Pérez TS, Nevárez-Moorillón GV, Ochoa-Velasco CE, Navarro-Cruz AR, Hernández-Carranza P, Avila-Sosa R. The relation between drying conditions and the development of volatile compounds in saffron (Crocus sativus). *Molecules.* (2021) 26:6954. doi: 10.3390/molecules26226954

8. Mirhadi E, Nassirli H, Malaekeh-Nikouei B. An updated review on therapeutic effects of nanoparticle-based formulations of saffron components (safranal, crocin, and crocetin). *J Pharmaceutical Invest.* (2020) 50:47–58. doi: 10.1007/s40005-019-00435-1

9. Allahdad Z, Khammari A, Karami L, Ghasemi A, Sirotkin VA, Haertlé T, et al. Binding studies of crocin to β-Lactoglobulin and its impacts on both components. *Food Hydrocoll.* (2020) 108:106003. doi: 10.1016/j.foodhyd.2020.106003

10. Ali A, Mughal H, Ahmad N, Babar Q, Saeed A, Khalid W, et al. Novel therapeutic drug strategies to tackle immune-oncological challenges faced by cancer patients during COVID-19. *Exp Rev Anticancer Ther.* (2021) 21:1371–83. doi: 10.1080/14737140.2021.1991317

11. Ali A, Manzoor MF, Ahmad N, Aadil RM, Qin H, Siddique R, et al. The burden of cancer, government strategic policies, and challenges in pakistan: a comprehensive review. *Front Nutr.* (2022) 9:1–17. doi: 10.3389/fnut.2022.940514

12. Bastani S, Vahedian V, Rashidi M, Mir A, Mirzaei S, Alipourfard I, et al. An evaluation on potential anti-oxidant and anti-inflammatory effects of crocin. *Biomed Pharmacother.* (2022) 153:113297. doi: 10.1016/j.biopha.2022.113297

13. Shahidani S, Rajaei Z, Alaei H. Pretreatment with crocin along with treadmill exercise ameliorates motor and memory deficits in hemiparkinsonian rats by anti-inflammatory and antioxidant mechanisms. *Metab Brain Dis.* (2019) 34:459–68. doi: 10.1007/s11011-018-0379-z

14. Feigin VL, Vos T, Nichols E, Owolabi MO, Carroll WM, Dichgans M, et al. The global burden of neurological disorders: translating evidence into policy. *Lancet Neurol.* (2020) 19:255–65. doi: 10.1016/S1474-4422(19)30411-9

15. Cho KS, Shin M, Kim S, Lee SB. Recent advances in studies on the therapeutic potential of dietary carotenoids in neurodegenerative diseases. *Oxidative Med Cell Long.* (2018) 2018:1–13. doi: 10.1155/2018/4120458

16. Ahmed S, Hasan MM, Heydari M, Rauf A, Bawazeer S, Abu-Izneid T, et al. Therapeutic potentials of crocin in medication of neurological disorders. *Food Chem Toxicol.* (2020) 145:111739. doi: 10.1016/j.fct.2020.111739

17. Kazemi F, Vosough I, Sepahi S, Mohajeri SA. Effect of crocin versus fluoxetine in treatment of mild to moderate obsessive-compulsive disorder: a double blind randomized clinical trial. *Human Psychopharmacol.* (2021) 36:e2780. doi: 10.1002/hup.2780

18. Amanpour A, Kelebek H, Selli S. GLC/HPLC methods for Saffron (L.). *Bioactive Mol Food*. (2019) 1987–2035. doi: 10.1007/978-3-319-78030-6_42

19. Bathaie SZ, Farajzade A, Hoshyar R. A review of the chemistry and uses of crocins and crocetin, the carotenoid natural dyes in saffron, with particular emphasis on applications as colorants including their use as biological stains. *Biotechnic Histochem.* (2014) 89:401–11. doi: 10.3109/10520295.2014.890741

20. Alavizadeh SH, Hosseinzadeh H. Bioactivity assessment and toxicity of crocin: a comprehensive review. *Food Chem Toxicol.* (2014) 64:65–80. doi: 10.1016/j.fct.2013.11.016

21. Ni Y, Li L, Zhang W, Lu D, Zang C, Zhang D, et al. Discovery and LC-MS characterization of new crocins in gardeniae fructus and their neuroprotective potential. *J Agric Food Chem.* (2017) 65:2936–46. doi: 10.1021/acs.jafc.6b03866

22. Hussein RA, Salih NA, Eman Thabit N. Bioactivity of crocin pigment of saffron plant. *Plant Arch.* (2018) 18:357–64. Available online at: http://plantarchives.org/PDF%20181/357-364__PA3_4074_.pdf

23. Huang H, Zhu Y, Fu X, Zou Y, Li Q, Luo Z. Integrated natural deep eutectic solvent and pulse-ultrasonication for efficient extraction of crocins from gardenia fruits (Gardenia jasminoides Ellis) and its bioactivities. *Food Chem.* (2022) 380:132216. doi: 10.1016/j.foodchem.2022.132216

24. Singla RK, Bhat G. Crocin: an overview. *Indo Global J Pharmaceut Sci.* (2011) 1:281–6. doi: 10.35652/IGJPS.2011.27

25. Zeka K, Ruparelia KC, Continenza MA, Stagos D, Vegliò F, Arroo RR. Petals of Crocus sativus L. as a potential source of the antioxidants crocin and kaempferol. *Fitoterapia.* (2015) 107:128–34. doi: 10.1016/j.fitote.2015.05.014

26. Catinella G, Borgonovo G, Dallavalle S, Contente ML, Pinto A. From saffron residues to natural safranal: valorization of waste through a β-glucosidase. *Food Bioprod Proc.* (2022) 131:144–8. doi: 10.1016/j.fbp.2021.11.002

27. Cardone L, Castronuovo D, Perniola M, Cicco N, Candido V. Saffron (Crocus sativus L.). the king of spices: an overview. *Sci Horticult.* (2020) 272:109560. doi: 10.1016/j.scienta.2020.109560

28. Mashmoul M, Azlan A, Yusof BNM, Khaza'ai H, Mohtarrudin N, Boroushaki MT. Effects of saffron extract and crocin on anthropometrical, nutritional and lipid profile parameters of rats fed a high fat diet. *J Funct Foods.* (2014) 8:180–7. doi: 10.1016/j.jff.2014.03.017

29. Garrett H. *Shrubs and "Sort of" Shrubs. Plants for Houston and the Gulf Coast.* Austin, TX: University of Texas Press (2021). p. 75–100.

30. Defilipps RA, Krupnick GA. The medicinal plants of Myanmar. *PhytoKeys.* (2018) 1:1–341. doi: 10.3897/phytokeys.102.24380

31. Hashemi M, Hosseinzadeh H. A comprehensive review on biological activities and toxicology of crocetin. *Food Chem Toxicol.* (2019) 130:44–60. doi: 10.1016/j.fct.2019.05.017

32. Liqin T, Haocheng L, Jing W, Yujuan X, Wenni T, Lu L, et al. Study on ultrahigh-pressure extraction technology on properties of yellow extract from gardenia fruit. *J Food Composit Analysis.* (2021) 104:104186. doi: 10.1016/j.jfca.2021.104186

33. Ali A, Riaz S, Sameen A, Naumovski N, Iqbal MW, Rehman A, et al. The disposition of bioactive compounds from fruit waste, their extraction, and analysis using novel technologies: a review. *Processes.* (2022) 10:2014. doi: 10.3390/pr10102014

34. Prado JM, Veggi PC, Náthia-Neves G, Meireles MA. Extraction methods for obtaining natural blue colorants. *Curr Analyt Chem.* (2020) 16:504–32. doi: 10.2174/1573411014666181115125740

35. Qi Y, Zhang H, Liang S, Chen J, Yan X, Duan Z, et al. Ispitivanje antidepresivnog učinka funkcionalnog napitka s aktivnim peptidima, mentolom i eleuterozidom, te mehanizmi njegovog djelovanja u mišjem modelu. *Food Technol Biotechnol.* (2020) 58:295–302. doi: 10.17113/ftb.58.03.20.6568

36. Kupnik K, PrimoŽič M, Kokol V, Leitgeb M. Nanocellulose in drug delivery and antimicrobially active materials. *Polymers.* (2020) 12:2825. doi: 10.3390/polym12122825

37. Wang W, He P, Zhao D, Ye L, Dai L, Zhang X, et al. Construction of Escherichia coli cell factories for crocin biosynthesis. *Microb Cell Fact.* (2019) 18:1–11. doi: 10.1186/s12934-019-1166-1

38. Rahaman A, Kumari A, Farooq MA, Zeng X-A, Hassan S, Khalifa I, et al. Novel extraction techniques: an effective way to retrieve the bioactive compounds from saffron (Crocus Sativus). *Food Rev Int.* (2021) 10:1–29. doi: 10.1080/87559129.2021.1967377

39. Manzoor MF, Hussain A, Naumovski N, Ranjha MM, Ahmad N, Karrar E, et al. A narrative review of recent advances in rapid assessment of anthocyanins in agricultural and food products. *Front Nutr.* (2022) 9:1–14. doi: 10.3389/fnut.2022.901342

40. Garcia-Vaquero M, Rajauria G, Tiwari B. Conventional extraction techniques: solvent extraction. *Sust Seaweed Technol.* (2020) 171–89. doi: 10.1016/B978-0-12-817943-7.00006-8

41. Garavand F, Rahaee S, Vahedikia N, Jafari SM. Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients. *Trends Food Sci Technol.* (2019) 89:26–44. doi: 10.1016/j.tifs.2019.05.005

42. Vernès L, Vian M, Chemat F. Ultrasound and microwave as green tools for solid-liquid extraction. *Liquid Phase Extract.* (2020) 355–74. doi: 10.1016/B978-0-12-816911-7.00012-8

43. Manzoor MF, Hussain A, Sameen A, Sahar A, Khan S, Siddique R, et al. Novel extraction, rapid assessment and bioavailability improvement of quercetin: a review. *Ultrason Sonochem.* (2021) 78:105686. doi: 10.1016/j.ultsonch.2021.105686

44. Asbahani A, Miladi K, Badri W, Sala M, Addi EA, Casabianca H, et al. Essential oils: From extraction to encapsulation. *Int J Pharm.* (2015) 483:220. doi: 10.1016/j.ijpharm.2014.12.069

45. Manzoor MF, Ahmad N, Ahmed Z, Siddique R, Zeng XA, Rahaman A, et al. Novel extraction techniques and pharmaceutical activities of luteolin and its derivatives. *J Food Biochem.* (2019) 43:e12974. doi: 10.1111/jfbc.12974

46. Lee KX, Shameli K, Yew YP, Teow S-Y, Jahangirian H, Rafiee-Moghaddam R, et al. Recent developments in the facile bio-synthesis of gold nanoparticles (AuNPs) and their biomedical applications. *Int J Nanomed.* (2020) 15:275. doi: 10.2147/IJN.S233789

47. Manzoor MF, Zeng X-A, Rahaman A, Siddeeg A, Aadil RM, Ahmed Z, et al. Combined impact of pulsed electric field and ultrasound on bioactive compounds and FT-IR analysis of almond extract. *J Food Sci Technol.* (2019) 56:2355–64. doi: 10.1007/s13197-019-03627-7

48. O'sullivan A, O'callaghan Y, O'grady M, Hayes, M, Kerry J, O'brien N. The effect of solvents on the antioxidant activity in Caco-2 cells of Irish brown seaweed extracts prepared using accelerated solvent extraction (ASE®). *J Funct Foods.* (2013) 5:940–8. doi: 10.1016/j.jff.2013.02.007

49. Sarfarazi M, Jafari SM, Rajabzadeh G. Extraction optimization of saffron nutraceuticals through response surface methodology. *Food Anal Methods.* (2015) 8:2273–85. doi: 10.1007/s12161-014-9995-3

50. Goleroudbary MG, Ghoreishi S. Response surface optimization of safranal and crocin extraction from crocus sativus L. via supercritical fluid technology. *J Supercrit Fluids.* (2016) 108:136–44. doi: 10.1016/j.supflu.2015.10.024

51. Mohajeri SA, Hosseinzadeh H, Keyhanfar F, Aghamohammadian J. Extraction of crocin from saffron (Crocus sativus) using molecularly imprinted polymer solid-phase extraction. *J Sep Sci.* (2010) 33:2302–9. doi: 10.1002/jssc.201000183

52. Sarfarazi M, Jafari SM, Rajabzadeh G, Galanakis CM. Evaluation of microwave-assisted extraction technology for separation of bioactive components of saffron (Crocus sativus L.). *Industrial Crops Prod.* (2020) 145:111978. doi: 10.1016/j.indcrop.2019.111978

53. Manzoor MF, Hussain A, Tazeddinova D, Abylgazinova A, Xu B. Assessing the nutritional-value-based therapeutic potentials and non-destructive approaches for mulberry fruit assessment: an overview computational. *Intellig Neurosci.* (2022) 2022:1–16. doi: 10.1155/2022/6531483

54. Hadizadeh F, Mohajeri S, Seifi M. Extraction and purification of crocin from saffron stigmas employing a simple and efficient crystallization method. *Pakistan J Biol Sci.* (2010) 13:691–8. doi: 10.3923/pjbs.2010.691.698

55. Manzoor MF, Xu B, Khan S, Shukat R, Ahmad N, Imran M, et al. Impact of high-intensity thermosonication treatment on spinach juice: bioactive compounds, rheological, microbial, and enzymatic activities. *Ultrason Sonochem.* (2021) 78:105740. doi: 10.1016/j.ultsonch.2021.105740

56. Pham TQ, Cormier F, Farnworth E, Tong VH, Van Calsteren M-R. Antioxidant properties of crocin from Gardenia jasminoides ellis and study of the reactions of crocin with linoleic acid and crocin with oxygen. *J Agric Food Chem.* (2000) 48:1455–61. doi: 10.1021/jf991263j

57. Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI. HPLC quantification of major active components from 11 different saffron (Crocus sativus L.) sources. *Food Chem.* (2007) 100:1126–31. doi: 10.1016/j.foodchem.2005.11.020

58. Bakshi H, Sam S, Rozati R, Sultan P, Islam T, Rathore B, et al. DNA fragmentation and cell cycle arrest: a hallmark of apoptosis induced by crocin from kashmiri saffron in a human pancreatic cancer cell line. *Asian Pac J Cancer Prev.* (2010) 11:675–9. Available online at: http://journal.waocp.org/article_25263.html

59. Loskutov AV, Hong W-P, Sink KC. Biotechnology for the production of crocin in callus cultures of Gardenia jasminoides Ellis. *Plant Biol.* (2000) 2:161–5. Available online at: https://www.researchgate.net/profile/Andrey-Loskutov/publication/232318146_Biotechnology_for_the_production_of_crocin_in_callus_cultures_of_Gardenia_jasminoides_Ellis/links/0fcfd5081ed3a1232c000000/Biotechnology-for-the-production-of-crocin-in-callus-cultures-of-Gardenia-jasminoides-Ellis.pdf

60. Yang B, Liu X, Gao Y. Extraction optimization of bioactive compounds (crocin, geniposide and total phenolic compounds) from Gardenia (Gardenia jasminoides Ellis) fruits with response surface methodology. *Innovat Food Sci Emerg Technol.* (2009) 10:610–5. doi: 10.1016/j.ifset.2009.03.003

61. Iborra JOL, Castellar MR, Cánovas MA, Manjón AR. TLC preparative purification of picrocrocin, HTCC and crocin from saffron. *J Food Sci.* (1992) 57:714–6. doi: 10.1111/j.1365-2621.1992.tb08079.x

62. Bortolomeazzi R, Sebastianutto N, Toniolo R, Pizzariello A. Comparative evaluation of the antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential. *Food Chem.* (2007) 100:1481–9. doi: 10.1016/j.foodchem.2005.11.039

63. Yousefi-Nejad S, Heidarbeigi K, Roushani M. Electronic tongue as innovative instrument for detection of crocin concentration in saffron (Crocus sativus L.). *J Food Sci Technol.* (2022) 59:1–9. doi: 10.1007/s13197-021-05349-1

64. Zhang A, Shen Y, Cen M, Hong X, Shao Q, Chen Y, et al. Polysaccharide and crocin contents, and antioxidant activity of saffron from different origins. *Ind Crops Prod.* (2019) 133:111–7. doi: 10.1016/j.indcrop.2019.03.009

65. Heydari S, Haghayegh GH. Extraction and microextraction techniques for the determination of compounds from saffron. *Can Chem Trans.* (2014) 2:221–47. doi: 10.13179/canchemtrans.2014.02.02.0097

66. Kadkhodaee R, Hemmati-Kakhki A. Ultrasonic extraction of active compounds from saffron. *Int Symp Saffron Biol Technol.* (2006) 739:417–25. doi: 10.17660/ActaHortic.2007.739.55

67. Tong Y, Jiang Y, Guo D, Yan Y, Jiang S, Lu Y, et al. Homogenate extraction of crocins from saffron optimized by response surface methodology. *J Chem.* (2018) 2018:1–6. doi: 10.1155/2018/9649062

68. Sarfarazi M, Rajabzadeh Q, Tavakoli R, Ibrahim SA, Jafari SM. Ultrasound-assisted extraction of saffron bioactive compounds; separation of crocins, picrocrocin, and safranal optimized by artificial bee colony. *Ultrason Sonochem.* (2022) 86:105971. doi: 10.1016/j.ultsonch.2022.105971

69. Kyriakoudi A, Chrysanthou A, Mantzouridou F, Tsimidou MZ. Revisiting extraction of bioactive apocarotenoids from Crocus sativus L. dry stigmas (saffron). *Anal Chim Acta.* (2012) 755:77–85. doi: 10.1016/j.aca.2012.10.016

70. Karasu S, Bayram Y, Ozkan K, Sagdic O. Extraction optimization crocin pigments of saffron (Crocus sativus) using response surface methodology and determination stability of crocin microcapsules. *J Food Measur Characteriz.* (2019) 13:1515–23. doi: 10.1007/s11694-019-00067-x

71. Sobolev AP, Carradori S, Capitani D, Vista S, Trella A, Marini F, et al. Saffron samples of different origin: an NMR study of microwave-assisted extracts. *Foods.* (2014) 3:403–19. doi: 10.3390/foods3030403

72. Manna L, Bugnone CA, Banchero M. Valorization of hazelnut, coffee and grape wastes through supercritical fluid extraction of triglycerides and polyphenols. *J Supercrit Fluids.* (2015) 104:204–11. doi: 10.1016/j.supflu.2015.06.012

73. Alehosseini A, Gómez-Mascaraque LG, Ghorani B, Lopez-Rubio A. Stabilization of a saffron extract through its encapsulation within electrospun/electrosprayed zein structures. *Lwt.* (2019) 113:108280. doi: 10.1016/j.lwt.2019.108280

74. De Monte C, Cesa S. *Use of Saffron as a Functional Food and Saffron Nutraceuticals*. Saffron: Elsevier (2021). p. 241–73.

75. Bhat NA, Wani IA, Hamdani AM, Gani A. Development of functional cakes rich in bioactive compounds extracted from saffron and tomatoes. *J Food Sci Technol.* (2022) 59:2479–91. doi: 10.1007/s13197-021-05267-2

76. Dabbagh Moghaddam A, Garavand F, Razavi SH, Dini Talatappe H. Production of saffron-based probiotic beverage by lactic acid bacteria. *J Food Measurem Characteriz.* (2018) 12:2708–17. doi: 10.1007/s11694-018-9888-z

77. Armellini R, Peinado I, Pittia P, Scampicchio M, Heredia A, Andres A. Effect of saffron (Crocus sativus L.) enrichment on antioxidant and sensorial properties of wheat flour pasta. *Food Chem.* (2018) 254:55–63. doi: 10.1016/j.foodchem.2018.01.174

78. Bakshi RA, Sodhi NS, Wani IA, Khan ZS, Dhillon B, Gani A. Bioactive constituents of saffron plant: extraction, encapsulation and their food and pharmaceutical applications. *Appl Food Res.* (2022) 100076:1–15. doi: 10.1016/j.afres.2022.100076

79. Mehrnia M-A, Jafari S-M, Makhmal-Zadeh BS, Maghsoudlou Y. Crocin loaded nano-emulsions: Factors affecting emulsion properties in spontaneous emulsification. *Int J Biol Macromol.* (2016) 84:261–7. doi: 10.1016/j.ijbiomac.2015.12.029

80. Rahaiee S, Shojaosadati SA, Hashemi M, Moini S, Razavi SH. Improvement of crocin stability by biodegradeble nanoparticles of chitosan-alginate. *Int J Biol Macromol.* (2015) 79:423–32. doi: 10.1016/j.ijbiomac.2015.04.041

81. Mehrnia M-A, Jafari S-M, Makhmal-Zadeh BS, Maghsoudlou Y. Rheological and release properties of double nano-emulsions containing crocin prepared with angum gum, arabic gum and whey protein. *Food Hydrocoll.* (2017) 66:259–67. doi: 10.1016/j.foodhyd.2016.11.033

82. Rahaiee S, Hashemi M, Shojaosadati SA, Moini S, Razavi SH. Nanoparticles based on crocin loaded chitosan-alginate biopolymers: antioxidant activities, bioavailability and anticancer properties. *Int J Biol Macromol.* (2017) 99:401–8. doi: 10.1016/j.ijbiomac.2017.02.095

83. Armellini R, Peinado I, Asensio-Grau A, Pittia P, Scampicchio M, Heredia A, et al. *In vitro* starch digestibility and fate of crocins in pasta enriched with saffron extract. *Food Chem.* (2019) 283:155–63. doi: 10.1016/j.foodchem.2019.01.041

84. Li D, Wu G, Zhang H, Qi X. Preparation of crocin nanocomplex in order to increase its physical stability. *Food Hydrocoll.* (2021) 120:106415. doi: 10.1016/j.foodhyd.2020.106415

85. Chalatashvili A, Khositashvili M, Khositashvili T, Gorgiladze M, Buishvili G. Processing of tincture production technology from various plant raw materials containing crocin. *Winemaking Theory Pract.* (2018) 3:3–7. doi: 10.13187/winem.2018.1.3

86. Fan M, Li N, Li Q, Li Y, Qian H, Zhang H, Rao Z, Wang L. Development of steamed bread fortified with gardenia fruit pomace: an evaluation of its bioactive compounds and quality characteristics. (2022) 1–26. doi: 10.2139/ssrn.4074755

87. Bajerska J, Mildner-Szkudlarz S, Podgórski T, Oszmatek-Pruszyńska E. Saffron (Crocus sativus L.) powder as an ingredient of rye bread: an anti-diabetic evaluation. *J Med Food.* (2013) 16:847–56. doi: 10.1089/jmf.2012.0168

88. Shvachko NA, Loskutov IG, Semilet TV, Popov VS, Kovaleva ON, Konarev AV. Bioactive components in oat and barley grain as a promising breeding trend for functional food production. *Molecules.* (2021) 26:2260. doi: 10.3390/molecules26082260

89. Ritota M, Comitato R, Manzi P. Cow and ewe cheeses made with saffron: characterization of bioactive compounds and their antiproliferative effect in cervical adenocarcinoma (HeLa) and breast cancer (MDA-MB-231) cells. *Molecules.* (2022) 27:1995. doi: 10.3390/molecules27061995

90. Licón C, Carmona M, Molina A, Berruga M. Chemical microbiological, textural, color, and sensory characteristics of pressed ewe milk cheeses with saffron (Crocus sativus L) during ripening. *J Dairy Sci.* (2012) 95:4263–74. doi: 10.3168/jds.2012-5389

91. Aktypis A, Christodoulou ED, Manolopoulou E, Georgala A, Daferera D, Polysiou M. Fresh ovine cheese supplemented with saffron (Crocus sativus L.): impact on microbiological physicochemical, antioxidant, color and sensory characteristics during storage. *Small Ruminant Res.* (2018) 167:32–8. doi: 10.1016/j.smallrumres.2018.07.016

92. Almodóvar P, Prodanov M, Arruñada O, Inarejos-García AM Affron® eye. a natural extract of saffron (Crocus sativus L) with colorant properties as novel replacer of saffron stigmas in culinary and food applications. *Int J Gastronomy Food Sci.* (2018) 12:1–5. doi: 10.1016/j.ijgfs.2018.03.001

93. Arnold M, Rajagukguk YV, Gramza-Michałowska A. Functional food for elderly high in antioxidant and chicken eggshell calcium to reduce the risk of osteoporosis—a narrative review. *Foods.* (2021) 10:656. doi: 10.3390/foods10030656

94. Gani A, Jan R, Ashwar BA, Ul Ashraf Z, Shah A, Gani A. Encapsulation of saffron and sea buckthorn bioactives: Its utilization for development of low glycemic baked product for growing diabetic population of the world. *LWT.* (2021) 142:111035. doi: 10.1016/j.lwt.2021.111035

95. Delfanian M, Sahari MA. Improving functionality, bioavailability, nutraceutical and sensory attributes of fortified foods using phenolics-loaded nanocarriers as natural ingredients. *Food Res Int.* (2020) 137:109555. doi: 10.1016/j.foodres.2020.109555

96. Mzabri I, Addi M, Berrichi A. Traditional and modern uses of saffron (Crocus sativus). *Cosmetics.* (2019) 6:63. doi: 10.3390/cosmetics6040063

97. Arora P, Ansari S, Arora S. Nutritional beverages. *Am J Pharmatech Res.* (2019) 9:1–28. doi: 10.46624/ajptr.2020.v10.i3.015

98. Chrysanthou A, Pouliou E, Kyriakoudi A, Tsimidou MZ. Sensory threshold studies of picrocrocin, the major bitter compound of saffron. *J Food Sci.* (2016) 81:S189–98. doi: 10.1111/1750-3841.13152

99. Kyriakoudi A, Pouliou E, Ordoudi SA, Tsimidou S. Sensorial and functional attributes of herbal infusions containing saffron. 2nd Imeko. *Foods.* (2016) 304–9. Available online at: https://www.imeko.org/publications/tc23-2016/IMEKO-TC23-2016-061.pdf

100. Noori T, Sureda A, Sobarzo-Sánchez E, Shirooie S. The role of natural products in treatment of depressive disorder. *Curr Neuropharmacol.* (2022) 20:929–49. doi: 10.2174/1570159X20666220103140834

101. Tang J, Lu L, Wang Q, Liu H, Xue W, Zhou T, et al. Crocin reverses depression-like behavior in Parkinson disease mice via VTA-mPFC pathway. *Mol Neurobiol.* (2020) 57:3158–70. doi: 10.1007/s12035-020-01941-2

102. Abbaszadeh-Mashkani S, Hoque SS, Banafshe HR, Ghaderi A. The effect of crocin (the main active saffron constituent) on the cognitive functions, craving, and withdrawal syndrome in opioid patients under methadone maintenance treatment. *Phytother Res.* (2021) 35:1486–94. doi: 10.1002/ptr.6913

103. Hosseinzadeh H, Sadeghnia HR, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Effects of saffron (Crocus sativus L.) and its active constituent. crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. *Phytother Res.* (2012) 26:381–6. doi: 10.1002/ptr.3566

104. Duan X, Liu F, Kwon H, Byun Y, Minn I, Cai X, et al. (S)-3-(Carboxyformamido)-2-(3-(carboxymethyl) ureido) propanoic acid as a novel PSMA targeting scaffold for prostate cancer imaging. *J Med Chem.* (2020) 63:3563–76. doi: 10.1021/acs.jmedchem.9b02031

105. Papandreou MA, Tsachaki M, Efthimiopoulos S, Cordopatis P, Lamari FN, Margarity M. Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. *Behav Brain Res.* (2011) 219:197–204. doi: 10.1016/j.bbr.2011.01.007

106. Gol S, Pena RN, Rothschild MF, Tor M, Estany J. A polymorphism in the fatty acid desaturase-2 gene is associated with the arachidonic acid metabolism in pigs. *Sci Rep.* (2018) 8:1–9. doi: 10.1038/s41598-018-32710-w

107. Nam KN, Park Y-M, Jung H-J, Lee JY, Min BD, Park S-U, et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. *Eur J Pharmacol.* (2010) 648:110–6. doi: 10.1016/j.ejphar.2010.09.003

108. Naghizadeh B, Mansouri M, Ghorbanzadeh B, Farbood Y, Sarkaki A. Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats. *Phytomedicine.* (2013) 20:537–42. doi: 10.1016/j.phymed.2012.12.019

109. Srivastava S, Ahmad R, Khare SK. Alzheimer's disease and its treatment by different approaches: a review. *Eur J Med Chem.* (2021) 216:113320. doi: 10.1016/j.ejmech.2021.113320

110. Cenini G, Voos W. Mitochondria as potential targets in Alzheimer disease therapy: an update. *Front Pharmacol.* (2019) 10:902. doi: 10.3389/fphar.2019.00902

111. Ghoweri AO, Gagolewicz P, Frazier HN, Gant JC, Andrew RD, Bennett BM, et al. Neuronal calcium imaging, excitability, and plasticity changes in the Aldh2–/–mouse model of sporadic Alzheimer's disease. *J Alzheimer's Dis.* (2020) 77:1623–37. doi: 10.3233/JAD-200617

112. Ali A, Ain Q, Saeed A, Khalid W, Ahmed M, Bostani A. Biomolecular Characteristics of Whey Proteins with Relation to Inflammation. *IntechOpen*. (2021). p. 1–18. doi: 10.5772/intechopen.99220

113. Saito T, Saido TC. Neuroinflammation in mouse models of Alzheimer's disease. *Clin Exp Neuroimmunol.* (2018) 9:211–8. doi: 10.1111/cen3.12475

114. Latif A, Bibi S, Ali S, Ammara A, Ahmad M, Khan A, et al. New multitarget directed benzimidazole-2-thiol-based heterocycles as prospective anti-radical and anti-Alzheimer's agents. *Drug Dev Res.* (2021) 82:207–16. doi: 10.1002/ddr.21740

115. Wang C, Cai X, Hu W, Li Z, Kong F, Chen X, et al. Investigation of the neuroprotective effects of crocin via antioxidant activities in HT22 cells and in mice with Alzheimer's disease. *Int J Mol Med.* (2019) 43:956–66. doi: 10.3892/ijmm.2018.4032

116. Li J-Y, Li N-N, Wang L, Peng J-X, Duan L-R, Chen C-L, et al. A compound heterozygous PINK1-associated juvenile Parkinson's disease with pregnancy in Chinese. *J Neurol.* (2021) 268:2223–7. doi: 10.1007/s00415-021-10405-z

117. Raza C, Anjum R. Parkinson's disease: mechanisms, translational models and management strategies. *Life Sci.* (2019) 226:77–90. doi: 10.1016/j.lfs.2019.03.057

118. Mohammadzadeh L, Rahbardar MG, Razavi BM, Hosseinzadeh H. Crocin protects malathion-induced parkinson-like disease by inhibiting apoptosis and α-synuclein accumulation in rats'. *Striatum*. (2021) 1–21. doi: 10.21203/rs.3.rs-1028580/v1

119. El Midaoui A, Ghzaiel I, Vervandier-Fasseur D, Ksila M, Zarrouk A, Nury T, et al. Saffron (Crocus sativus L.): a source of nutrients for health and for the treatment of neuropsychiatric and age-related diseases. *Nutrients.* (2022) 14:597. doi: 10.3390/nu14030597

120. Pekna M, Stokowska A, Pekny M. Targeting complement C3a receptor to improve outcome after ischemic brain injury. *Neurochem Res.* (2021) 46:2626–37. doi: 10.1007/s11064-021-03419-6

121. Povroznik JM, Engler-Chiurazzi EB, Nanavati T, Pergami P. Absolute lymphocyte and neutrophil counts in neonatal ischemic brain injury. *SAGE Open Medicine.* (2018) 6:2050312117752613. doi: 10.1177/2050312117752613

122. Huang L, Zhang L. Neural stem cell therapies and hypoxic-ischemic brain injury. *Prog Neurobiol.* (2019) 173:1–17. doi: 10.1016/j.pneurobio.2018.05.004

123. Azami S, Shahriari Z, Asgharzade S, Farkhondeh T, Sadeghi M, Ahmadi F, et al. Therapeutic potential of saffron (Crocus sativus L) in ischemia stroke. *Evid Based Complem Alternat Med.* (2021) 2021:1–8. doi: 10.1155/2021/6643950

124. Beghi E. The epidemiology of epilepsy. *Neuroepidemiology.* (2020) 54:185–91. doi: 10.1159/000503831

125. Motaghinejad M, Safari S, Feizipour S, Sadr S. Crocin may be useful to prevent or treatment of alcohol induced neurodegeneration and neurobehavioral sequels via modulation of CREB/BDNF and Akt/GSK signaling pathway. *Med Hypotheses.* (2019) 124:21–5. doi: 10.1016/j.mehy.2019.01.017

126. Mazumder AG, Sharma P, Patial V, Singh D. Crocin attenuates kindling development and associated cognitive impairments in mice via inhibiting reactive oxygen species-mediated NF-κB activation. *Basic Clin Pharmacol Toxicol.* (2017) 120:426–33. doi: 10.1111/bcpt.12694

127. Barton N, D'errico F. North African origins of symbolically mediated behaviour and the Aterian. *Develop Quatern Sci.* (2012) 23–34. doi: 10.1016/B978-0-444-53821-5.00003-8

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Traditional food consumption pattern and nutritional status of Oraons: An Asian Indian indigenous community

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Introduction: Food consumption is an intensive social activity and can be considered a cultural artifact, reflecting the intricate process of sociocultural differentiation in shaping eating habits. Food has a positive effect on a person's health, physical performance, and state of mind. The nutritional composition of a person's diet plays a significant role in their overall health and development. Moreover, tribal cuisine is incomplete without a traditional drink. Rice beer, or *handia*, is one such indigenous alcoholic-fermented beverage that serves as a staple food.

Methods: This exploratory cross-sectional study was conducted from January, 2018 to December, 2020 to explore the traditional food practices of the Oraon community through a combination of one-to-one interviews, focus group discussions, and measurements. This study also involved the documentation of individual food recipes, from collection to preparation. To assess nutrient intake, we used a 24-h dietary recall method for 200 Oraon families comprising 466 adults and 193 children. Anthropometric measurements, such as body mass index (BMI) for adults and height-for-age and BMI-for-age z-score methods for children, recommended by the WHO, were taken and recorded using standard procedures. Additionally, the nutrient content of *handia* was analyzed.

Results: The mean (SD) height, weight, mid-upper arm circumference (MUAC), and BMI of the Oraon people were analyzed. The combined overall prevalence of chronic energy deficiency (CED) (BMI < 18.5 kg/m^2) was 39% (37% in men and 40.3% in women). The prevalence of overweight and obesity (BMI ≥ 25.0 kg/m^2) was found to be 7.1% among only women. The average daily energy intake was calculated to be 2,290 kcal per capita. It is worth noting that the food and nutrient consumption of the Oraon tribe was largely consistent with the recommended daily allowances/intakes (RDA/RDI). The consumption of root and tuber products was particularly high. The Oraon tribe was found to have a familiar intake of animal protein in the form of meat, including common periwinkles (*Littorina littorea*). The study also discovered a remarkable array of unique, region-specific festive foods. The consumption of nutrient-rich fermented rice beer was especially noteworthy.

Conclusion: The present study provides insight into the traditional food practices of the Oraon tribe in West Bengal. It also highlights that their indigenous food consumption patterns have undergone significant changes as a result of admixture with other communities. To address these dietary issues, it is recommended that regional, need-based planning, and effective intervention programs be implemented. To ensure the proper maintenance of the Oraon tribe's traditional food practices, the cultivation of kitchen gardens and the domestication of wild, edible plants, such as seeds and tubers, may be helpful. Moreover, promoting the consumption of macronutrient-fortified *handia,* an indigenous beverage with high medicinal benefits, could be effective in combating hidden hunger among adults.

KEYWORDS

Oraon, tribe, traditional, food, nutrition, *handia*, West Bengal

Introduction

Food consumption is an intensive social activity and can be considered a cultural artifact, reflecting the intricate process of sociocultural differentiation in shaping eating habits. Food has a positive effect on a person's health, physical performance, and state of mind. The nutritional composition of a person's diet plays a significant role in their overall health and development. When all nutrients are consumed in the appropriate proportion required by the body, it is known as "good" or "optimum" nutrition, which helps to maintain good health.

However, malnutrition, that is, undernourishment, micronutrient deficiencies, and obesity, is a result of an ongoing nutrition transition and poses a significant threat to public health (Pinstrup-Andersen, 2007; von Grebmer et al., 2014). Thus, studying food and eating habits is important as food plays a fundamental role in human survival.

Tribal communities are closely connected to nature and its resources, and this connection is reflected in their simple and respectful approach to food.

The same reverence is reflected in their cuisine; tribal communities consider their food sacred. Tribal cuisine is not only interesting but also nutritious and well-balanced. Tribal food systems are an integral part of their cultural heritage and traditions. Food is an important part of their identity and culture. It reflects the geography to which they belong and the locally available resources used in their cuisine.

The dietary patterns of the tribes in India living in various regions and agro-climatic conditions may vary greatly due to their secluded lifestyles, food habits, dietary practices, and attitudes toward food. Beliefs, customs, and traditions influence the general pattern of living in any community. It is well known that the geological composition of the soil determines the occurrence of local flora, cropping patterns, and the associated agricultural practices in a given area. Knowledge of the food resources available to humans has been crucial in allowing them to survive in adverse conditions.

As per the 2011 census, the total scheduled tribes in India make up 8.6% of the country's total population. The census records 705 different tribes, with 75 of them designated as particularly vulnerable tribal groups (PVTGs). West Bengal is the 9th-most highly populated state in India by tribes, accounting for 5.1% of the country's tribal population. Approximately 5.8% of the state's total population of 9.13 crores includes scheduled tribes (Bisai et al., 2014). In West Bengal, the total population of the Oraon tribe is 643,510, of which 322,933 are men and 320,577 are women. The sex ratio of the Oraon community is 993 women per 1,000 men. The literacy rate in the Oraon community is 59.0%. This rate is higher for men, at 68.1%, compared to that of women, which is 49.9% (Dutta and Bisai, 2020). The population of Oraon people has grown by 4.27% in the decade between 2001 and 2011. The Oraon tribe is a Dravidian-speaking agricultural community from Chhota Nagpur. They have their own language, Kurukh, and follow strict marriage customs that involve clan exogamy and tribe endogamy. The Oraon tribe practices strict exogamy within their clans and endogamy within the tribe. They have a rich cultural heritage and a multitude of exogamous septs such as Ikka, Minz, Kujur, Bura, Turkey, Beck, Khess, Bandh, Bakura, Bahula, Khakha, Tigga, Toppo, Lakra, Bakhla, Bando, Bara, Barwa or Khoea, Kerketta, Khalko, Kindo, Kispota, Munjini, Pana, and Runda, each with a unique totem that serves as a symbol of identity and is considered taboo by its members. With the aim of preserving the flavors of ingredients, they cook with very few spices and consume food that is mostly raw, semi-cooked, roasted, or fermented. Additionally, many members of the Oraon tribe have diversified into other occupations, including trade and commerce, and some have taken up professional jobs. However, agriculture remains a significant part of their livelihood and is deeply intertwined with their cultural identity. They consume non-vegetarian diet. Rice is their staple food, while mutton, fowl, fish, and eggs are eaten with great pleasure. They also consume some locally available pulses, green leafy vegetables, and vegetables with rice. Tea has gained popularity as a beverage. Haria (in Bengali) or Handia (in Hindi) plays an important role in its consumption in everyday life and on different occasions. Handia is prepared from parboiled rice and a mixture of fermented inoculums called *Ranu* or *Bakhor*. Geographical variations may create a distinction in the nutritional value of this fermented brew. In view of the above, the present study was undertaken to examine the impact of traditional food patterns on the nutritional health of the Oraon tribe, the second-largest tribal community in West Bengal, India.

Materials and methods

This exploratory cross-sectional study was conducted in six districts of West Bengal: Birbhum, Dakshin Dinajpur, Jalpaiguri (undivided), North 24 Parganas, Purulia, and Paschim Medinipur.

Abbreviations: BMI, body mass index; CED, chronic energy deficiency; CU, consumption unit; MUAC, mid-upper arm circumference; PC, per capita; PVTGs, particularly vulnerable tribal groups; RDA, recommended dietary allowance; RDI, recommended dietary intake; and GLV, green leafy vegetable.

TABLE 1 List of common food items consumed by Oraon people of West Bengal.

Food group	English name	Scientific name	Kurukh/ Oraon term	Seasonality	Food intake type
Cereal/grain	African Millet	*Eleusine coracana*	Kodai	All season	Process
	Barley	*Hordeum vulgare*	Yab	All season	Process
	Khoi	*Oryza sativa*	Irika	All season	Fry
	Maize or Corn	*Zea mays*	Jinhor	Rainy	Process
	Puffed rice/Muri	*Oryza sativa*	Irika tixil/Murhi	All season	Process
	Rice	*Oryza sativa*	Tixil	All season	Process
	Rice flacks	*Oryza sativa*	Chepte, Alkhara	All season	Process, Non-process
	Sorghum/Jorar	*Sorghum bicolor*	Jowar	All season	Process
	Semolina	*Triticum turgidum*	Adar	All Season	Process
	Tapioca seeds	*Manihot esculenta*	Sabugota	All season	Process
	Vermicelli	*Vermicellini*	Sewai	All season	Process
	Wheat flour (refined)	*Triticum aestivum*	Gohom gunda	All Season	Process
	Wheat flour (whole)	*Triticum aestivum*	Gohom gunda	All season	Process
Pulses	Arher Dal	*Cajanus cajan*	Rahri	All season	Process
	Bengal gram whole	*Cicer arietinum*	Boot	All season	Process, Non-process
	Bengal gram	*Cicer arietinum*	Boot	All season	Process
	Besan (Gram flour)	-	Boot gunda	All season	Process
	Black gram (whole)	*Vigna mungo*	Maasi	All season	Process
	Field bean seeds	*Vicia faba*	Beangota	Winter	Process
	Green gram	*Vigna radiata*	Hariyar boot	All season	Process
	Khesari Dal	*Lathyrus sativus*	Khesari daali	All season	Process
	Lentils	*Lens esculenta*	Kensa	All season	Process
	Soyabean	*Glycine max Merr.*	Seya	All season	Process
Vegetables	Bamboo	*Bambusoideae*	Bans	All season	Process
	Beet	*Beta vulgaris*	Xenso murai	Winter	Process, Non process
	Bitter gourd	*Momordica charantia*	Karela	All season	Process
	Bottle gourd	*Lagenaria siceraria*	Lauwa	Winter	Process
	Brinjal	*Solanum melongena*	Bhetango	All season	Process
	Broad beans	*Vicia faba*	Simbi	Winter	Process
	Capsicum	*Capsicum annum*	Kapsikam	Winter	Process
	Carrot	*Daucus carota*	Xenso murai gajar	Winter	Process, Non-process
	Cauliflower	*Brassica oleracea var. botrytis*	Kubi	Winter	Process
	Colocasia stem	*Colocasia esculenta*	Kisgo/Pechki donre	Rainy	Process
	Cow Peapods	*Vigna unguiculata*	Oye batar choppa	Rainy	Process
	Cucumber	*Cucumis sativas*	Palxanja	Rainy, Winter	Non process
	Drums stick	*Moringa oleifera*	Munga donrey	Rainy	Process
	Drums stick flower	*Moringa oleifera*	Munga poomp	Rainy, Winter	Process
	Elephant foot yam	*Amorphophallus paeoniifolius*	Koha sakhin	All season	Process
	French Beans	*Phaseolus vulgaris*	Kat simbi	Winter	Process
	Jack fruits seeds	*Artocarpus heterophyllus*	Kathar kowa	Summer	Process

(Continued)

TABLE 1 (Continued)

Food group	English name	Scientific name	Kurukh/ Oraon term	Seasonality	Food intake type
	Jack fruits Tender	*Artocarpus heterophyllus*	Kathar (bolo)	Summer	Process
	Ladies Finger	*Abelmoschus esculenta*	Bhrewa	Summer, Rain	Process
	Lotus stem	*Nelumbo nucifera*	Purni donrey	On collection	Process
	Mango green	*Mangifera indica*	Tatxa (xeyna)	Summer	Process, Non process
	Mushroom	*Agaricusbisporus*	Oosa	Rainy	Process
	Onion	*Allium cepa*	Peyanch	All Season	Process, Non-process
	Papaya green	*Carica papaya*	Papita (xeyna)	All season	Process
	Parwar	*Trichosanthes dioica*	Parwal	Winter	Process
	Plantain Flower	*Plantago major*	Kera poomp	On collection	Process
	Pea green	*Pisum sativum*	Hariyar batar	Winter	Process
	Plantain Green	*Plantago major*	Kera (hariyar/xeyna)	On collection	Process
	Plantain Stem	*Plantago major*	Kera xosga	On collection	Process
	Potato	*Solanum tuberosum*	Aluwa	All season	Process
	Pumpkin	*Cucurbita moschata*	Tumba/Konhra	All season	Process
	Radish	*Raphanus sativus*	Rasri, Nasri	Winter	Process, Non-process
	Ridge Gourd	*Luffa*	Konhra	Rainy	Process
	Snake Gourd	*Trichosanthescucumerina*	Chihnga	All season	Process
	Spinney yam	*Diascorea esculenta*	Kisgo, aru	All season	Process
	Sweet Potato	*Ipomoea batatas*	Sakar kanda	All season, winter	Process
	Tomato	*Solanum lycopersicum*	Bilaichi/Bhejri	All season	Process, Non-process
	Turnip	*Brassica rapa*	Salgam	Winter	Process
	Yam	*Dioscorea*	Pechki	Rainy	Process
Leafy vegetables	Amaranth	*Amaranthus virdis*	Arkha	Rainy	Process
	Bathua Leaves	*Chenopodium album*	Puchchhu arxa	Rainy	Process
	Bottle gourd Leaves	*Lagenaria siceraria*	Tumba arxa	Rainy	Process
	Cabbage	*Brassica oleracea var. capitata*	Atxa kubi	Winter	Process
	Cauliflower leaves	*Brassica oleracea*	Pomp kubi	Winter	Process
	Colocosia Leaves	*Colocasia esculenta*	Kisgo/ Pichke atxa	Rainy	Process
	Coriander Leaves	*Coriander sativum*	Dhaniya atxa	Rainy	Process
	Fenugreek Leaves	*Tribonellafoenum-graecum*	Methi arkha	Winter	Process
	Mustard Leaves	*Brassica juncea*	Mani arxa	Rainy	Process
	Pumpkin Leaves	*Cucurbita moschata*	Tumba arxa	Rainy	Process
	Radish Leaves	*Raphanus sativus*	Murai arxa	Rainy	Process
	Spinach	*Spinacia oleracea*	Palak arxa	Rainy	Process
	Susni Sag	*Marsilea quadrifolia Linn*	Suinsuin arxa	Rainy	Process
	Turnip Greens	*Brassica rapa*	Gutand murai arxa	Rainy	Process
	Tea Flower	*Camellia sinensis*	Chah poomp	Winter	Process
	Bata	*Labeobata*	Padaru injo	All season	Process
	Chingri	*Dendrobranchiate*	Choppo	All season	Process
	Crab	*Brachyura*	Kakro	All season	Process
	Hilsa	*Tenualosailisha*	Hilsa	Rainy	Process

(Continued)

TABLE 1 (Continued)

Food group	English name	Scientific name	Kurukh/ Oraon term	Seasonality	Food intake type
Fish	Katla	*Catlacatla*	Koha jhila injo	All season	Process
	Khoyra	*Sardine*	Khiira	All season	Process
	Koi	*Cyprinus rubrofuscus*	Kusma	All season	Process
	Koocha Machli	-	Chanachka injo	All season	Process
	Lata	*Channa punctatus*	Leta	All season	Process
	Mackrel	*Scomberscombrus*	Gujali	All season	Process
	Magur	*Clariasbatrachus*	Maigra	All season	Process
	Mrigal	*Cirrhinuscirrhosus*	Koha xensoxann injo	All season	Process
	Pabda	*Ompokbimaculatus*	Choyen Malka injo	All season	Process
	Pangas	*Pangasius*	Pengas	All season	Process
	Pomfrets	*Bramidae*	Pomfret	All season	Process
	Puti	*Puntius sophore*	Puthi	All season	Process
	Rohu	*Labeorohita*	Bilchina injo	All season	Process
	Singhi	*Heteropneustesfossilis*	Maigra	All season	Process
	Sole	*Soleasolea*	Chhuddi	All season	Process
	Sorputi	*Puntius*	Koha puthi	All season	Process
	Tapsi	*Polynemusparadiseus*	Tatxa injo	All season	Process
	Telapia	*Oreochromis niloticus*	Isung injo	All season	Process
	Tengra	*Sperataseenghala*	Tengra	All season	Process
Meat	Beef	*Bos taurus*	Addo ahra	All season	Process
	Chicken	*Gallus gallusdomesticus*	Xeyr ahra	All season	Process
	Duck meat	*Anatidae*	Gerey ahra	All season	Process
	Egg	*Gallus gallus*	Bee	All season	Process
	Goat	*Capra aegagrus hircus*	Eyra	All season	Process
	Monitor lizard	*Varanus*	Guinh tetenga	On hunting	Process
	Pork	*Sus scrofa domesticus*	Kiss ahra	All season	Process
	Rabbit	*Oryctolagus cuniculus*	Kharha, muyan	All season	Process
	Snail/periwinkles	*Littorina littorea*	Ghonghi	On collection	Process
Milk	Cow Milk	*Bos taurus*	Oye dudhi	All season	Process
	Milk Powder	-	Paudardudhi	All season	Process
Spices	Black Mustard	*Brassica nigra*	Lutni/moxaaro mani	All season	Process
	Black pepper	*Piper nigrum*	Moxaaro maircha	All season	Process
	Cardamom	*Elettaria cardamomum*	Jira	All season	Process
	Clove	*Syzygiumaromaticum*	Lawang	All season	Process
	Coriander	*Coriander sativum*	Dhaniya	All season	Process
	Dry chili	*Capsicum annuum*	Xaika maircha	All season	Process
	Fennel	*Foeniculum vulgare*	Ond kita gahi xoppa	All season	Non process
	Garlic	*Allium sativum*	Rasri	All season	Process
	Ginger	*Zingiber officinale*	Adxi	All season	Process
	Myrobalan	*Terminalia chebula*	aonra	All season	Process
	Turmeric	*Curcuma longa*	Baalka	All season	Process

(Continued)

TABLE 1 (Continued)

Food group	English name	Scientific name	Kurukh/ Oraon term	Seasonality	Food intake type
Sugar	Honey	*Apis cerana indica*	Tiini	All season	Non process
	Jaggery	-	Gulley	Winter	Non process
	Sugars	-	Chini	All season	Process
Beverage	Handia	-	Jharaa, boryey	All season	Process (Fermented)
	Tea	*Camellia sinesis*	Chahamm	All season	Process

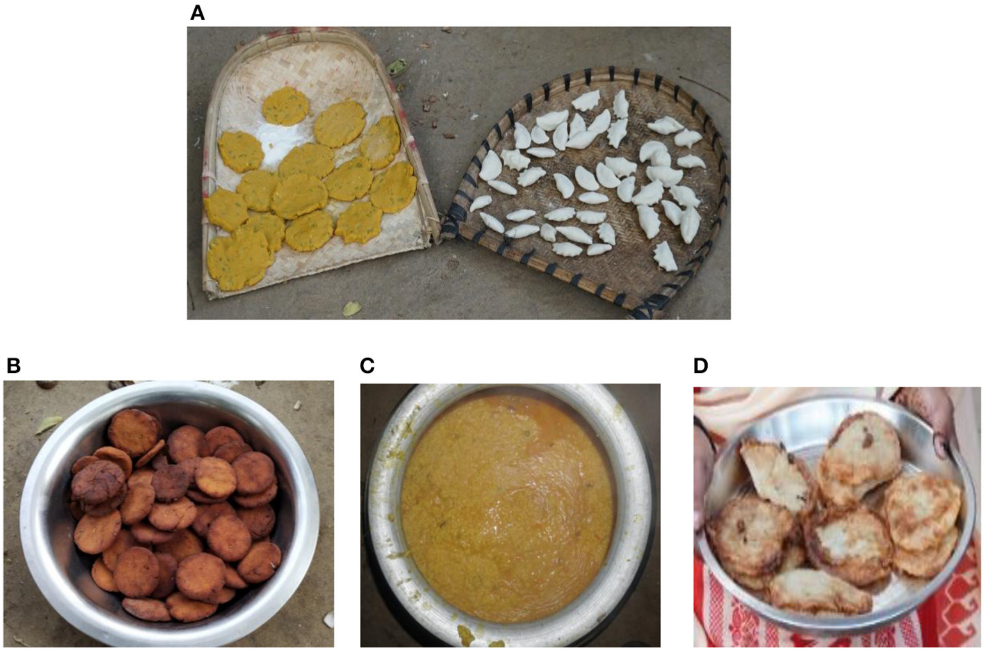

FIGURE 1
Festive foods: **(A)** Different types of *pitha (cake).* **(B)** Gur-pitha. **(C)** Bhog is made for god. **(D)** Aske-pitha.

The tribal villages in these districts were strategically selected based on their high concentration of Oraon communities, as well as their diverse geographical settlements and variations. This study aimed to document the role of indigenous foods in promoting good nutrition and balanced eating habits among the Oraon tribal community in West Bengal. Fieldwork for this study was carried out from January, 2018 to December, 2020. To accurately compare the CED between men and women in the studied community, the minimum sample size was calculated using a standard formula:

$$n = \frac{2 \times \overline{p}\left(100 - \overline{p}\right) \times \left(Z_{\alpha} + Z_{\beta}\right)^{2}}{\left(p1 - p2\right)^{2}}$$

The prevalence of CED among men and women was 47% and 31%, respectively (Mittal and Srivastava, 2006). With a 95% confidence interval and 80% power, a minimum estimated sample size was 146 from each group. Therefore, a total of 200 households were selected through a simple random sampling method for food analysis and assessment of nutritional status. Anthropometric data were collected from 466 adults (men = 203 and women = 233) and 193 children. Informed consent was obtained from all the participants before data collection. All data were collected by trained investigators.

Primary data on commonly consumed Indigenous food items were collected through interviews with a pre-validated questionnaire and focus group discussions. The questionnaire underwent a pilot survey before the main study was conducted to ensure its accuracy and effectiveness (Bisai and Dutta, 2021a). These data were used to assess the extent of food options available to the Oraon community and evaluate the significance of wild, indigenous foods in their regular diets. The 24-h dietary recall method was used to collect data on food and nutrient consumption. The tribal households were contacted through their respective district administrations. Most of the respondents were homemakers with extensive knowledge and experience in food preparation techniques. All recipes were collected through audio-visual methods while the female member of the household was cooking food.

The food item information was recorded by its most common English name, its availability during certain seasons, and how it was consumed. The items were grouped together based on their edible part, providing an organized description. The nutritional value of each food item was determined using the method outlined by Gopalan et al. (1989).

The total carbohydrate content of *handia* was determined using the Anthrone method (Yemm and Willis, 1954). The level of glucose was estimated using the standard biochemical method: the DNS method (Lv et al., 2021). Protein content was estimated using Lowry's method (Ledoux and Lamy, 1986). Moreover, fat content was quantified using the standard extraction-titration method (Frankel and Tarassuk, 1955). The level of alcohol was determined according to the colorimetric method described by Sumbhate et al. (2012). All the chemicals used in this experiment—Anthrone, DNS, Folin–Ciocalteu reagent, and others—were of analytical grade and purchased from Himedia and SRL in India. Distilled water was used in all the experiments. The instruments used in the study were a microprocessor-based UV-VIS double-beam spectrophotometer (model LI-2700) and a μP photocolorimeter (S. No. 17010015), both of which were purchased from Haryana, India.

Anthropometric characteristics such as height, weight, and mid-upper arm circumference (MUAC) were measured using standard methods (Lohman et al., 1988). The research personnel underwent comprehensive training to ensure accurate measurement techniques were used as part of the multi-pass strategy. Adult nutritional status was assessed using the BMI cutoffs recognized internationally (WHO., 1995). The children's nutritional status was evaluated using height-for-age and body mass index-for-age z-scores, calculated using the WHO Anthro-Plus software. Stunting and thinness were classified as Z-score values less than the −2 standard deviation of the reference median. All statistical analyses were performed using SPSS and MedCalc software. A *p*-value of <0.05 was considered statistically significant.

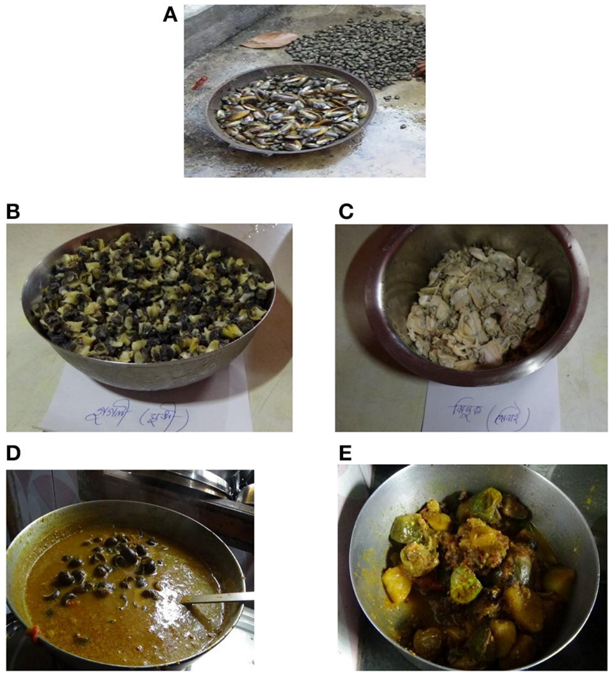

FIGURE 2
Protein rich foods: **(A)** The *Ghungi* (Common Periwinkles). **(B, C)** Meat of periwinkles. **(D, E)** Common periwinkles being cooked in a different way.

FIGURE 3
Seasonal foods: **(A)** Cooked tea flower. **(B)** Raw (preserved) tea flower.

Results

The food practices of indigenous communities, such as the Oraon tribe, are characterized by their rich cultural traditions and the utilization of locally available resources and techniques. The population under study also displays their unique dietary patterns in various geographical locations. While the food values of many indigenous foods, such as plants, insects, and fungi, have been explored (DeFoliart, 1992; Boa, 2004; Kuhnlein et al., 2009; Rathode, 2009), there is still a lack of research on the nutrient intake pattern of the tribes in India. The present study aimed to explore the food habits of the Oraon tribal community, particularly with respect to their varied geographical habitat, use of, nutritional value, and traditional knowledge of indigenous foods.

A list of commonly consumed food items by the Oraons is presented in Table 1. The dietary practices of the Oraon community, which resides in a diverse ecological area, are mentioned in the following text. Data on the recipes were gathered from the interviews and recorded as part of the documentation process.

The Oraon community of the Purulia district cohabits with other communities, leading to significant changes in their eating habits. Rice is the staple food for the Oraon community. They generally obtain vegetables from nearby markets. Due to the rocky terrain of the adjacent hills, they are unable to collect any wild food. They only collect the *jeerhol* flower in March and April and consume it on the day of their *nabanna* festival. They eat *khichdi* on the day of their *Sarul puja*, a seasonal festival. The Oraon tribe residing in Paschim Medinipur has a history of migration from the Chhota Nagpur region dating back several generations. They refer to themselves as the heirs of those who helped Lord Rama fight Ravana. They celebrate *Goyal puja* (worshiping the cowshed) in *Kartik* (from October to November). They prepare a special dish made from *Dhoan moong dal* (yellow lentils) and chicken that has been offered as a sacrifice at the cowshed. On the same day, they prepare a dish called *khichdi* with rice and biri dal, which they offer to the cow after worshiping it (except for

TABLE 2 Anthropometric characteristics of adults Oraons of West Bengal.

Anthropometry	Sex	*n*	Mean	SD	*t*-value
Height (cm)	Men	203	160.6	6.5	18.611
	Women	263	149.5	6.3	$P < 0.001$
Weight (kg)	Men	203	50.5	7.9	8.075
	Women	263	44.2	8.5	$P < 0.001$
MUAC (cm)	Men	203	25.5	2.8	6.934
	Women	263	23.6	3.1	$P < 0.001$
BMI (kg/m2)	Men	203	19.5	2.7	0.791
	Women	263	19.8	3.6	$P > 0.05$

TABLE 3 Nutritional status based on BMI (WHO., 1995) of adult Oraons of West Bengal.

Nutritional status	Men	Women	Total
CED Grade-III	14 (6.9)	26 (9.9)	40 (8.6)
CED Grade-II	20 (9.9)	35 (13.3)	55 (11.8)
CED Grade-I	41 (20.2)	45 (17.1)	86 (18.5)
Normal	120 (59.1)	132 (50.2)	252 (54.1)
Overweight	8 (3.9)	23 (8.7)	33 (6.7)
Obese	0	2 (0.8)	2 (0.4)
Total	203	263	466

pregnant cows). During the *Agrahan–Poush* months (November to January), when the new harvest is brought into their homes, they celebrate the *nabanna* festival with different types of *pitha* (rice cake), especially *mangshopitha* (made from rice powder and chicken) (Figure 1A). On the last day of *Poush* month (mid-January), they celebrate *Poush Sankranti*. They make *poushkush* or *pooshladdu* with rice powder and fill them with coconut crumbs, sesame, ginger, jaggery, or sugar. On the occasion of *Rash Purnima* in the *Kartik–Agrahan* (November) month, they make *gur pithas* (Figure 1B), which they distribute to their relatives. While visiting the nearby forest in the rainy season, if found, they collect Kham aalu, Kurkuri Chhatu, Bon kundri, cashew nuts, and yams. They consume *handia* daily, including at festivals. The Oraon tribe residing in the Birbhum district reveres "Nagpur-Dhanpur" as their chief god, which they worship in the Chhota Nagpur region of Jharkhand. The Oraon community receives a letter each year listing the names of those who are required to attend a yearly pilgrimage to the Chhota Nagpur region of Jharkhand. During this pilgrimage, it is mandatory for participants to wear traditional clothing and speak in their mother tongue, Kurukh, also known as *Thaar Bhasa*. Each clan has its own puja, called the *Basanti Puja*, which takes place every 10 years. During the year when the Basanti Puja is held, no marriages are arranged within that particular year. They sacrifice four kinds of animals for this puja: *boroboli*, *mejoboli*, *sejoboli,* and *chhotoboli*. A fully grown-up buffalo with large horns is sacrificed as *boroboli*. A pig is sacrificed as *mejoboli*. A male goat is sacrificed as *sejoboli,* and a hen is sacrificed as *chhotoboli*. They observe a 24-h long fast, which they break with *bhog* made from rice and a sacrificed hen (Figure 1C). While celebrating the *Karam puja* in the *Bhadra* month (from August to September), they offer three hens of different colors as a sacrifice. The meat is then cooked with rice without the use of any oil. The red hen, or *khayer,* and the *tamakatu* hen are consumed by the male members of the village, while the white hen is consumed by the female members of the village. The head and leg portions are reserved for the elders, referred to as *morobbi*. On the occasion of *Poushsankranti*, they prepare various types of pitha (Figure 1A), including a rice cake made with *kalokolai* paste. On the day of *poilaMagh* (mid-January), they celebrate their *Shalgram puja*, during which they prepare *askepithe* (Figure 1D) using rice powder and jaggery. They cook it in an earthen pot. It is customary that postpartum mothers do not receive any cooked food after giving birth. They are allowed to eat only *muri* (puffed rice) or chiwda and drink lukewarm water. For up to 21 days after delivery, they can only have boiled food without any spices or oil once a day. Consumption of meat, fish, eggs, or fruits is strictly prohibited during this postpartum pollution period. After the *Nokhna* ceremony, on the 21st day after childbirth, the mother is allowed to enter the kitchen and resume her normal diet. The consumption of *Paatsaak* (jute leaves) and periwinkles (a species of small edible whelks) is a common dietary habit among the Oraons community of Dakshin Dinajpur (Figures 2A–E). They celebrate *Ashari Maayer Pujo* in the month of *Ashar* (from June to July) with the belief that it will bring prosperity through rainfall. The ritual of *Dandakatta* holds significance in this festival. The Oraon people perform it before any ceremony or to purify the house. The main occupation of the Oraon people residing in the Jalpaiguri and Alipurduar districts is tea gardening. The main issue with the Oraon people in this region is that most families adopted Christianity as their religion due to the inclusion of missionaries. Thus, they have undergone many cultural changes. Food patterns have also changed. Only the consumption of tea flowers could be found among them, which solely depends on availability (Figure 3A). They preserve it for longer use (Figure 3B). It tastes bitter and is cooked with tomatoes and other vegetables. The Oraon tribe in the North 24 Parganas district of West Bengal has adapted the tropical climate of mangrove area in this delta region. They cook the *shalukful* (water lily), *bunoamra* (wild mombins), and *keora* fruit. They catch rodents and cook them with different spices.

Table 2 summarizes the anthropometric characteristics expressed as the mean and standard deviation of weight, height, MUAC, and BMI of adult Oraon men and women. As

TABLE 4 Average foodstuff consumption (g/pc/day) of Oraons of West Bengal.

Food intake	RDA (ICMR 1990)	West Bengal (NNMB, Tribal 2009)	Birhor (Bisai and Dutta, 2021a)	Lodha (Bisai and Dutta, 2021b)	Toto (Bisai and Dutta, 2021c)	Oraon (present study)
Cereals	460	610.4	396.2	378.5	401.5	477.2
Pulses	40	10.1	46.7	66.7	34.8	19.0
GLV	40	77.7	39.1	38.3	42.6	18.7
Other vegetables	60	44.0	73.6	76.5	91.8	48.4
Roots and tubers	50	86.1	227.4	182.9	320.2	223.5
Milk	150	1.8	2.9	-	25.8	5.0
Fat and oils	40	7.7	18.5	16.9	34.1	11.4
Sugar and jaggery	30	3.7	7.6	4.7	11.4	3.4

TABLE 5 Average nutrient consumption (pc/day) of Oraons of West Bengal.

Nutrient intake	RDA (ICMR 1990)	West Bengal (NNMB, Tribal 2009)	Birhor (Bisai and Dutta, 2021a)	Lodha (Bisai and Dutta, 2021b)	Toto (Bisai and Dutta, 2021c)	Oraon (present study)
Energy (kcal)	2,425	2,303	1,934	1,727	2,175	2,290
Protein (g)	60	50.2	50.2	43.9	59.9	56.0
Fat (g)	20	10.6	23.1	18.8	36.9	17.3
Calcium (mg)	400	195.0	155.2	140.4	231.2	323.1
Iron (mg)	28	11.1	9.2	7.1	12.7	13.8
Thiamine (mg)	1.2	1.5	0.7	0.6	0.8	1.7
Riboflavin (mg)	1.4	0.4	0.4	0.4	0.4	1.5
Niacin (mg)	16	24.5	12.1	10.8	12.2	23.4
Vitamin-C (mg)	40	37.8	62.1	57.7	65.5	65.6
Folic acid (μg)	200	61.4	84.4	76.3	86.2	72.1

expected, mean weight, height, and MUAC were significantly higher among men than women. The nutritional status of the adult Oraons of West Bengal is presented in Table 3. The overall prevalence of CED and overweight/obesity was 39% (men: 37% and women: 40.3%) and 7%, respectively. It was observed that the prevalence of CED and overweight/obesity among Oraon women is higher than that among men. Moreover, the prevalence of stunting and thinness (CED) among children aged under 18 years was found to be 34.2 and 32.6%, respectively.

It was revealed that *handia* consumption improves the nutritional status of men and women, and there is a relationship between the nutritional status of mothers and infants. However, the present data are not sufficient, and further research is required. Table 4 summarizes the average food intake in a day for the Oraon tribe. Comparing the present study with other national-level tribal studies, we observed that the Oraon tribe consumes a good number of tubers, vegetables, and green leafy vegetables. Their average food intake seems adequate, and it is in accordance with the RDA, except for milk, fat, oil, sugar, and jaggery.

Table 5 displays the average daily nutrient intake (CU/day) of an adult Oraon compared to other studies conducted in West Bengal (NNMB., 2009; Bisai and Dutta, 2021a,b,c) and the RDA. It is worth noting that energy intake among the Oraon tribe is better than that of the PVTGs (Birhor, Lodha, and Toto) of West Bengal; however, it falls below the RDA.

Handia, a rice-based fermented drink, is integral to their lives. They consume *handia* on every occasion. It has medicinal and nutritional benefits. The analysis of the nutrient composition of raw (before mixing water) *handia* is presented in Table 6. The analysis shows that raw *handia* has a high protein content (162 mg/100 ml), followed by carbohydrates (55 mg/100 ml), alcohol (11.3%), glucose (10.2 mg/100 ml), and fat (5.6 mg/100 ml). The appearance of raw and consumable *handia* is depicted in Figures 4A, B.

Discussion

The rich knowledge of indigenous food practices among tribal communities in India highlights a wide range of cultural diversity, traditions, and environments (International Fund for Agricultural Development., 2003; Singh et al., 2007). The fascinating use of natural foods in Indian tribal communities highlights the diversity of their cultural traditions and environments. This knowledge has the potential to improve food security, nutrition, and health. Indigenous tribes hold a colossal responsibility for preserving 80% of the world's biodiversity (FAO., 2021). Their traditional food systems are important not only for cultural, social, and economic reasons but also for preserving their cuisine and way of life, as well as maintaining their cultural heritage within the community (Bhat, 2012; Durst and Bayasgalanbat, 2014). Tribal communities cultivate a variety of food crops, including a few uncommon ones. They also preserve wild foods such as mushrooms, bamboo shoots, caryota, palm pith, and so on (Rajyalakshmi, 1991). A recent study found that the Oraon community is aware of indigenous varieties of green leafy vegetables (GLVs) and consumes them during certain months or throughout the year (Ghosh-Jerath et al., 2018). Other indigenous food items from different food groups, such as cereals, roots and tubers, other vegetables, mushrooms, fruits, meat and fish, oil, and alcoholic drinks, were also reported. Fermented rice beer is a nutritious and energy-boosting beverage that is widely recognized among indigenous people worldwide but is mainly found in a few tropical Asian countries, including India. It has been reported that rice is a good source of carbohydrates (77–89%) and energy (350–475 kcal) (FAO., 1993). The fermentation process makes rice more nutritious as microbes partially digest the substrate into simple sugar and facilitate the bioavailability of lactic acid, alcohol, minerals, and bioactive compounds. Handia is a cheap, high-calorie, mild, fermented alcoholic beverage made from broken rice and consumed as a staple food by the ancestral and low-income communities of lateritic West Bengal, Assam, Bihar, Orissa, Jharkhand, and different areas of eastern and central India (Ghosh et al., 2014). Earlier studies reported that the alcohol and sugar content of raw fermentation increases with the prolongation of the fermentation time due to the presence of yeast that produces alcohol. This is achieved through the process of anaerobic fermentation, where the yeast converts simple sugars into alcohol. This increase in alcohol and sugar content is observed after the mixture has been diluted with drinking water. By the time it is consumed, the alcohol content reduces to only 2–3% (Ghosh et al., 2014, 2015). It has been well documented that the parts of plants used to prepare *handia* may increase the shelf-life of microbes by acting as preservatives. Moreover, the parts of the plant contain many bioactive compounds (Mallavadhani et al., 2004; Manikandan and Doss, 2010). Handia is used as a starter culture by tribal people in the tropical region, including India. Moreover, haria is a rice-based fermented beverage that is consumed as a staple food by the tribal people of central and eastern India (Ghosh et al., 2015).

There are several health benefits of the lactic acid content of *handia*, such as immunostimulation, cholesterol reduction, increased endocrine secretion, stress relief, and brain stimulation. It also helps protect overall intestinal function (Cory Holly Institute., 2005). In this study, the Oraon people consumed, on average, 50 ml of *handia* daily, which highlights the cultural significance of this traditional beverage in their community. The adult male member of the family consumes a good amount of *handia* before going to work. School-aged children also consume *handia* occasionally, primarily during festivals and marriage ceremonies. Currently, scholarly interest in rice-based fermented products is growing globally due to their high caloric value, accessibility, and widespread acceptance among the general population. Handia holds a notable cultural significance among the indigenous communities of India, who view it as an essential part of their dietary culture, survival, and maintenance of good health. The promotion of macronutrient-fortified *handia* may be an effective means of combating hidden hunger among adult indigenous people in India.

TABLE 6 Nutrient content of *handia* (raw).

Sl. no.	Parameters tested	Results
1	Carbohydrate	55 mg/100 ml
2	Glucose	10.2 mg/100 ml
3	Protein	162 mg/100 ml
4	Fat	5.6 mg/100 ml
5	Alcohol	11.3%

FIGURE 4
Fermented food: **(A)** Raw *handia*. **(B)** Consumable *handia* in a bottle with other festive foods.

A recent systematic review reported that approximately 45% and 49% of men and women, respectively, in India suffered from CED (Dutta et al., 2021). The present study found an overall prevalence of CED and overweight/obesity of 39 and 7%, respectively. A previous study found that 47% of males and 30.7% of females suffer from CED (Mittal and Srivastava, 2006). Another study reported 39.2% CED among Oraon women from Jharkhand (Ghosh-Jerath et al., 2018). Another study from the Alipurduar district of West Bengal revealed that the prevalence of CED was 34.5% in men and 53.5% in women (Bhattacharya et al., 2019). The present study found the prevalence of malnutrition, as indicated by stunting and thinness, was comparatively lower among Oraon children compared to adults and children of other tribal communities in the state (Bisai et al., 2010; Das et al., 2012).

The present study highlights the considerable consumption of tubers, vegetables, and green leafy vegetables by the Oraon community. However, several factors, such as geographical limitations, limited access to agricultural technology, sociocultural practices, and community conditions, may contribute to poor nutrition and health outcomes in these communities (Bhattacharjee et al., 2009). Indigenous communities continue to face food shortages and poor diets, leading to chronic diseases (Egeland and Harrison, 2013). The lack of diversity and nutrient density in their diets has been identified as a major concern. Mittal and Srivastava (2006) reported that the diets of all Oraon tribal populations in West Bengal were deficient in all food groups, with women and children particularly vulnerable to undernourishment. The minimal consumption of milk and fruits further highlights their susceptibility to protein-energy malnutrition (Bisai and Dutta, 2021b). This lack of essential nutrients in their diets (protein, vitamins, and iron) results in poor nutritional status and a negative impact on their overall health (Gole, 2015). However, the Oraon community consumes indigenous foods that are rich sources of micronutrients, namely calcium, iron, vitamin A, and folic acid (Ghosh-Jerath et al., 2015, 2018). It has been well documented that the nutritional status of Oraon tribal adults and children in Sambalpur, Orissa, was not satisfactory, with all the children suffering from different levels of malnutrition (Beck and Mishra, 2011). Studies across the globe have suggested that there has been a shift in dietary patterns, a phenomenon known as "nutrition transition" (Popkin, 2006). In present times, many tribal communities, especially those with tourist attractions, are experiencing a shift toward westernized diets instead of their traditional diets due to the influence of modern society and the growth of "ethno-tourism" (Dutta, 2014). The shift has resulted in a significant change in the indigenous pattern of food consumption among the Oraon community due to the mixing of other communities.

In conclusion, effective intervention programs should be implemented based on regional needs. Effectively maintaining kitchen gardens and domesticating wild edible plants, seeds, tubers, and so forth may help improve nutrition. Moreover, the promotion of macronutrient-fortified rice-based beverages such as *handia* may be useful in combating hidden hunger among adults, as it is an indigenous beverage with high calorie content and medicinal properties.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was conducted according to ICMR guidelines and informed consent was obtained from each participant to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Beck, P., and Mishra, B. K. (2011). Anthropometric profile and nutritional status of selected Oraon tribals in and around Sambalpur town, Orissa. *Stud. Tribes Tribals.* 9, 1–9. doi: 10.1080/0972639X.2011.11886623

Bhat, S. (2012). *Importance of Traditional Food System* 1st Edition Chapter 2. Tumakuru: The Registrar, Tumkur University. 4–10.

Bhattacharjee, L., Kothari, G., Priya, V., and Nandi, B. K. (2009). "The Bhil food system: Links to food security, nutrition and health," in *Indigenous people's food systems: The many dimensions of culture, diversity and environment for nutrition and health,* eds. H. V. Kuhnlein, B. Erasmus, D. Spigelski (Rome, Italy: FAO) 209–230.

Bhattacharya, A., Mukherjee, S., and Roy, S. K. (2019). Nutritional assessment of Oraons of West Bengal: a comparison between biochemical and anthropometric methods. *Anthropol. Rev.* 82, 297–311. doi: 10.2478/anre-2019-0022

Bisai, S., and Dutta, S. (2021a). "Food consumption and nutritional status of Birhor tribe of West Bengal," in *4th International Health Sciences and Innovation Congress, Azerbaijan, Liberty Publications, USA* 409–423.

Bisai, S., and Dutta, S. (2021b). "Traditional food practices of Lodha: a gathering-hunting indigenous community of West Bengal, India," in *3rd International Conference on Food, Agriculture and Veterinary* (Izmir-Turkey). 510–519.

Bisai, S., and Dutta, S. (2021c). "Food practices and nutritional status of toto community: an indigenous group of West Bengal, India," in *1st International Marmara Scientific Research and Innovation Congress* (Istanbul, Turkey, IKSAD Publishing House) 140–151.

Bisai, S., Ghosh, T., De, G. K., and Bose, K. (2010). Very high prevalence of thinness among Kora-Mudi tribal children of Paschim Medinipur District of West Bengal, India. *eJ. Biol. Sci.* 3, 43–49.

Bisai, S., Saha, K. B., Sharma, R. K., Muniyandi, M., and Singh, N. (2014). An overview of tribal population in India. *Tribal Health Bull.* 20, 1–126.

Boa, E. (2004). *Wild Edible Fungi: A Global Overview of Their Use and Importance to People: Series on Nonwooden Forest Products.* Rome, Italy: United Nations.

Cory Holly Institute. (2005). Lactic Acid: Friend or Foe? Available online at: www.coryholly.com/articles/article.cfm?id=35 (accessed January 2014).

Das, S., Mahata, M., and Bose, K. (2012). Age-trend in thinness among birhor children and adolescents of Purulia: a primitive tribe of West Bengal, India. *Asian J. Biol. Life Sci.* 1, 58–60.

DeFoliart, G. (1992). Insects as human food. *Crop. Protec.* 11, 395–399. doi: 10.1016/0261-2194(92)90020-6

Durst, P., and Bayasgalanbat, N. (2014). "Regional Symposium on "Promotion of underutilized indigenous food resources for food security and nutrition in Asia and the Pacific", KhonKaen, Thailand, 31 May-2 June 2012," in *FAO Regional Office for Asia and the Pacific Maliwan Mansion,* eds. P. Durst (Thailand: FAO Regional Office for Asia and the Pacific).

Dutta, R. (2014). *Concept of health, disease and treatment among the Totos of Totopara in Jalpaiguri district, West Bengal.* Doctoral Thesis, Department of Anthropology. University of North Bengal.

Dutta, S., and Bisai, S. (2020). Literacy trends and differences of scheduled tribes in West Bengal: a community level analysis. *Online J. Anthropol.* 16, 25–32.

Dutta, S., Biswas, S., and Bisai, S. (2021). Sex variation of chronic energy deficiency. *Online J. Anthropol.* 17, 241–259.

Egeland, G. M., and Harrison, G. G. (2013). "Health disparities: Promoting indigenous peoples' health through traditional food systems and self-determination," in *Centre for Indigenous peoples' Nutrition and Environment (CINE), School of Dietetics and Human Nutrition,* eds. G. M. Egeland (Montreal, Quebec, Canada: Food and Agriculture Organization of the United Nations (FAO)).

FAO. (1993). "Rice in human nutrition," in *FAO Food and Nutrition Series*, eds. B.O. Juliano (Los Banos, Laguna, Philippines: International Rice Research Institute (IRRI)) 35–48.

FAO. (2021). The Global-hub on Indigenous Food System. Available online at: http://www.fao.org/indigenous-peoples/global-hub/en/ (accessed November 20, 2022).

Frankel, E., and Tarassuk, N. P. (1955). An extraction-titration method for the determination of free fatty acids in rancid milk and cream. *J. Dairy Sci.* 38, 751–763. doi: 10.3168/jds.S0022-0302(55)95036-0

Ghosh, K., Maity, C., Adak, A., Halder, S. K., Jana, A., Das, A., et al. (2014). Ethnic Preparation of Haria, a Rice-Based Fermented Beverage, in the Province of Lateritic West Bengal, India. *Ethnobot. Res. Appl.* 12, 039–049.

Ghosh, K., Ray, M., Adak, A., Dey, P., Halder, S. K., Das, A., et al. (2015). Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage. *Food Chem.* 168, 196–202. doi: 10.1016/j.foodchem.2014.07.042

Ghosh-Jerath, S., Singh, A., Kamboj, P., Goldberg, G., and Magsumbol, M. S. (2015). Traditional knowledge and nutritive value of indigenous foods in the Oraon tribal community of Jharkhand: an exploratory cross-sectional study. *Ecol. Food. Nutr.* 54, 493–519. doi: 10.1080/03670244.2015.1017758

Ghosh-Jerath, S., Singh, A., Lyngdoh, T., Magsumbol, M. S., Kamboj, P., and Goldberg, G. (2018). Estimates of indigenous food consumption and their contribution to nutrient intake in Oraon tribal women of Jharkhand, India. *Food Nutr. Bull.* 39, 581–594. doi: 10.1177/0379572118805652

Gole, U. (2015). Nutritional status of the Oraon tribes of Jashpur District, Chhattisgarh. *Int. J. Sci. Res. Pub.* 5, 91–100.

Gopalan, C., Sastri, B. V. R., and Balasubramanian, S. C. (1989). *Nutritive Value of Indian Foods*. Hyderabad, India: National Institute of Nutrition.

International Fund for Agricultural Development. (2003). *Indigenous peoples and sustainable development.* United Nation: IFAD.

Kuhnlein, H. V., Erasmus, B., and Spigelski, D. (2009). *Indigenous Peoples' Food Systems: The Many Dimensions of Culture, Diversity and Environment for Nutrition and Health.* Rome: FAO.

Ledoux, M., and Lamy, F. (1986). Determination of proteins and sulfobetaine with the folin-phenol reagent. *Anal. Biochem.* 157, 28–31. doi: 10.1016/0003-2697(86)90191-0

Lohman, T. G., Roche, A. F., and Martorell, R. (1988). *Anthropometric Standardization Reference Manual.* Chicago, Ill, USA: Human Kinetics Books.

Lv, X., Wang, P., Wang, T., Zhao, J., and Zhang, Y. (2021). Development and validation of an improved 3-methyl-2-benzothiazolinone hydrazone method for quantitative determination of reducing sugar ends in chitooligosaccharides. *Food Chem.* 343, 128532. doi: 10.1016/j.foodchem.2020.128532

Mallavadhani, U. V., A., Mahapatra, K., Jamil, and Reddy, P. S. (2004). Antimicrobial activity of some pentacyclictriterpenes and their synthesized 3-O-Lipophilic chains. *Biol. Pharm. Bull.* 27, 1576–1579. doi: 10.1248/bpb.27.1576

Manikandan, A., and Doss, D. V. A. (2010). Evaluation of biochemical contents, nutritional value, trace elements, SDSPAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). *J. Chem. Pharm. Res.* 2, 295–303.

Mittal, P. C., and Srivastava, S. (2006). Diet, nutritional status and food related traditions of Oraon tribes of New Mal (West Bengal), India. *Rural Remote Health.* 6, 385. doi: 10.22605/RRH385

NNMB. (2009). *Diet and Nutritional Status of Tribal Population and Prevalence of Hypertension among Adults - Report on Second Repeat Survey, National Institute of Nutrition* – ICMR, Hyderabad. Technical Report No, 25.

Pinstrup-Andersen, P. (2007). Agricultural research and policy for better health and nutrition in developing countries: a food systems approach. *Agric. Econ.* 37, 187–198. doi: 10.1111/j.1574-0862.2007.00244.x

Popkin, B. M. (2006). Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. *Am. J. Clin. Nutr.* 84, 289–298. doi: 10.1093/ajcn/84.2.289

Rajyalakshmi, P. (1991). *Tribals Food Habits.* New Delhi: Gian Publishing House.

Rathode, M. (2009). Nutrient content of important fruit trees from arid zone of Rajasthan. *J. Hortic. Forestry.* 1, 103–108.

Singh, R. K., Singh, A., and Sureja, A. K. (2007). Traditional foods of Monpa tribe of West Kameng, Arunachal Pradesh. *Indian J. Tradit. Know.* 6, 37–41.

Sumbhate, S. V., Nayak, S., Goupale, D., Tiwari, A., and Jadon, R. S. (2012). Colorimetric method for the estimation of ethanol in alcoholic-drinks. *J. Anal. Technol.* 1, 1–6.

von Grebmer, K., Saltzman, A., Birol, E., et al. (2014). *Global Hunger Index: The Challenge of Hidden Hunger*. Bonn, Washington, DC: Welthungerhilfe, International Food Policy Research Institute, and Concern Worldwide.

WHO. (1995). *Physical Status: The Use and Interpretation of Anthropometry.* Technical Report Series No. 854. Geneva: World Health Organization.

Yemm, E. W., and Willis, A. (1954). The estimation of carbohydrates in plant extracts by Anthrone. *Biochem. J.* 57, 508. doi: 10.1042/bj0570508