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

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

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# Editorial: Dietary change strategies for sustainable diets and their impact on human health, volume II

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Editorial on the Research Topic
Dietary change strategies for sustainable diets and their impact on human health, volume II

Promoting sustainable diets is crucial for both personal health and the wellbeing of the planet. To mitigate the environmental impact of our food system, which is closely tied to international health and our sustainability goals, implementing dietary-change strategies presents a viable solution to address this issue. Projections indicate a continued shift toward more sustainable diets on a global scale in the coming decades, with a concurrent positive effect on human health (Davies et al., 2023).

Our current global challenge is to promote and facilitate healthy, well-balanced diets for an estimated 10 billion people by 2050 (FAO et al., 2023). Recently, there has been a growing interest in innovative and sustainable approaches, such as incorporating plant-based ingredients or exploring alternatives like algae, single-cell protein, and insects. Sustaining the popularity of these ingredients requires the development of diets that are not only sustainable and nutritious but also replicate the sensory experience - including taste and texture - of familiar products, such as animal-derived ones. While transitioning to healthier, primarily plant-based diets is crucial for achieving our environmental targets, these shifts must navigate potential obstacles like economic factors (e.g., corruption, infrastructure), political considerations (e.g., ideology, values), social aspects (e.g., technology, lack of community support, social norms), and cultural influences (e.g., tradition, culture, religion) (Alcorta et al., 2021).

Nutritionists advocate for a shift toward increased consumption of healthier, primarily plant-based or plant-rich diets, as a viable alternative to meat-based diets. These dietary choices have the potential to offer significant benefits in terms of both public health and environmental impact

(Pointke and Pawelzik, 2022; Shabir et al., 2022). Meat-based diets tend to exert a greater environmental strain compared to plant-based ones, contributing to issues like natural resource depletion, particularly through extensive water use in livestock production, as well as substantial consumption of other resources, and pollution of both water and air (Espinosa-Marrón et al., 2022).

A healthy and balanced diet, as defined by the WHO, plays a crucial role in safeguarding against malnutrition in all its forms, as well as non-communicable diseases (NCDs) like diabetes, heart disease, stroke, and cancer (FAO and WHO, 2019; Ruthsatz and Candeias, 2020). Our health is intricately linked to our dietary choices, and it is common for individuals to not consistently adhere to a lifelong healthy diet. This is often influenced by factors such as the widespread availability of processed foods and shifts in our overall lifestyle. As part of WHO's prioritized initiatives, outlined in May 2018 and ratified in the 13th General Programme of Work (GPW13), WHO's focus has transitioned toward advocating for healthy lifestyles and promoting overall wellbeing for all. Presently, WHO lends support to the promotion of a conducive food environment – including food systems that encourage a diverse, balanced, and healthy diets. The goal of a balanced diet is to furnish our bodies with all essential nutrients, and achieving an optimal ratio between different food groups is pivotal in this endeavor (Cena and Calder, 2020).

Nearly all diets trend comes with distinct drawbacks - they may either completely eliminate a specific nutrient or promote foods associated with a notably low energy supply, often becoming more costly (Hargreaves et al., 2021). Prioritizing health is a pivotal consideration when contemplating dietary adjustments. Simultaneously, there is a growing demand for plant-based foods driven by heightened awareness of the environmental impact of meat consumption (Socol et al., 2022). The Food and Agriculture Organization (FAO) asserts that adopting a diverse, balanced, and nutritious diet is crucial for ensuring sustainable ecological, economic, and social food supply. A sustainable diet focuses not only on the nutritional aspects of food, but also on the environmental, social, and ethical implications of the ingredients chosen (Fidan et al., 2022). This means considering factors like where and how the food was produced, its impact on biodiversity, the use of natural resources, and the welfare of workers involved in the production process. The trend diets appear to have a significantly positive impact on human health. These diets can be adaptable and customizable to accommodate individual food preferences, availability, cultural practices, and socioeconomic values. By using food efficiently, individuals can ensure they have a diverse and balanced diet (Noort et al., 2022).

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## Conflict of interest

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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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# Sustainability and scalability of egg consumption in Burkina Faso for infant and young child feeding

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**Introduction:** Malnutrition is a significant challenge to the health of women and children in Burkina Faso. Given the critical role of animal source food on the health of infants and young children (IYC), interventions continue to explore the potential for eggs to prevent malnutrition.

**Methods:** Using data from the Un Oeuf intervention, which significantly increased IYC egg consumption, combined with quantitative and qualitative data from endline and 3-month follow-up, we explore the barriers and facilitating factors to IYC egg consumption and the sustainability and scalability of the intervention.

**Results:** Child egg consumption was high at follow-up in the Control, Partial, and Full Intervention arms (83.3, 88.2%, and 100, respectively). The Full Intervention arm had the highest mean number of eggs consumed (2.9, 2.6, and 5.7), which reflected a slight reduction from endline (6.2). All participants owned chickens at follow-up (100%), however, flock size varied. The Full Intervention arm had more chickens (mean 8.8) than the Control (5.1) or Partial Intervention (6.2) arms, which was a 50% reduction in below endline (18.5 chickens). Qualitative results indicate that chicken ownership, education about the nutritional value of eggs, and spousal support facilitated IYC egg consumption. Barriers included egg production, cultural taboos, and animal health. Motivational factors reported included the observed improvement in child health, increased availability of mothers' time, and mothers' financial independence. Knowledge sharing within the Full and Partial Intervention groups was widely reported, and the sustainability of IYC egg consumption was reinforced by accountability among mothers and to community leaders, flipbooks distributed during the project, and high motivation.

**Discussion:** Main findings indicate that mothers who received the full Un Oeuf intervention were able to overcome barriers to feeding their child an egg daily, were able to improve their livelihood, were motivated to continue feeding their child eggs, and saw the addition of eggs into the child's diet as sustainable. Future nutrition sensitive agriculture interventions should consider tailoring this approach for other LMIC contexts. Future research is needed to explore a possible threshold in the number of household chickens necessary to continuously feed a child an egg a day.

KEYWORDS

**nutrition, sustainability, animal source foods, child health, behavior change**

## Introduction

Animal source foods (ASF) have been identified by the World Health Organization (WHO) as the best high-quality, nutrient-rich food for children aged between 6 and 23 months (WHO, 2014). Due to their critical importance in the growth and development of infants and young children, several studies have sought to examine the impact of ASF consumption on child growth and development in low- and middle-income countries (LMIC) (1–4). Results in meta-analysis are varied and inconclusive in understanding the full impact of ASF on child growth and development, with authors indicating that the variability in study design and inconsistency in definitions of exposure (ASF consumption type, quantity, etc.) and outcomes (stunting, wasting, underweight, etc.) inhibit quantitative analysis across studies (5). The nutritional value of ASF, specifically eggs, is distinctly suited to support early child growth and development (6–8); thus, research continues to explore the use of ASF to improve the growth and development of children during key windows of development (9), particularly those in LMIC. One such study, the *Un Oeuf* study, was a cluster randomized controlled trial conducted in Burkina Faso (10). The *Un Oeuf* study tested a behavior change strategy geared toward increasing egg consumption in infants and young children (IYC) and found significant increases in both egg consumption and improvements in child growth outcomes (11). Given the near-ubiquitous nature of poultry in small-scale farming households within LMIC, this work aims to understand both the sustainability and scalability of the behavior change intervention implemented through the *Un Oeuf* study. Additionally, a follow up study was conducted 3 months after the completion of the *Un Oeuf* study to assess facilitating factors and child egg consumption. This paper examines follow-up data from the *Un Oeuf* endline and follow- up study, to better understand the motivation and experience of the participants in the original *Un Oeuf* study and examine the sustainability and scalability of the behavior change intervention to increase ASF consumption in IYC.

## Background

Burkina Faso is a low-income country in the Sahel region of Sub-Saharan Africa (SSA) with numerous poor development indicators, including high mortality rates among children under five (CU5), neonates, and infants (12). A significant challenge to child health in Burkina Faso is malnutrition—with high rates of malnutrition (8.1% wasting), anemia (77%), and stunting (26.1%) in CU5 (13, 14). Much of this is attributable to high levels of food insecurity, inadequate complementary feeding practices, poor dietary diversity, and a general lack of food availability (15).

In Burkina Faso, ASF consumption is low particularly among women and children (16, 17). Barriers to ASF consumption, such as cultural beliefs and stigma surrounding egg consumption by children, may significantly constrain the consumption of ASF and in particular chicken eggs in Burkina Faso (1, 18). Many studies have shown the importance of including livestock derived ASF (milk, meat, dairy, and eggs) in a child's diet, especially during the critical window of child development from conception through 2 years old (9, 19–21). Regular consumption of ASF has been shown to improve the growth, nutritional status, cognitive development, and overall health of a child (22). Additionally, there is evidence showing the essential role mothers play in improving childhood nutrition (23, 24), and there is growing evidence that targeting and empowering female caregivers of children through livestock production and programming may improve child nutrition through increased ASF consumption (11, 25).

Within Burkina Faso, livestock is typically produced for income, gifting, and socio-religious practices, as opposed to production for direct, household consumption among household members (10). Consequently, innovative approaches that encourage, facilitate, and ultimately increase ASF consumption among rural livestock holders are needed. Using successful seminal egg studies from Ecuador (1) and Ethiopia (26) as guides, the *Un Oeuf* study aimed to increase ASF consumption in IYC through an innovative intervention that involved the gifting of chickens by a community champion and a culturally tailored behavior change strategy to improve livestock production and empower women (10, 11). Results from the intervention showed a significant increase egg consumption (11). Due to the high level of behavior change observed prior to the end of the intervention, additional funding was sought and secured to add a qualitative research component and a three-month follow-up survey to examine the facilitating factors and barriers to egg consumption within the context of the study. Leveraging these FGD and survey data this study aims to (1) examine and describe the facilitating factors that allowed for behavior change observed in the *Un Oeuf* study, and (2) examine the sustainability and scalability of the behavior change intervention to increase ASF consumption in IYC.

# Materials and methods

## Study setting

This research was conducted in 18 rural villages in the Kaya region of Burkina Faso. Villages were in a resource poor setting and comprised of households whose livelihoods were based on smallholder farming. Households in these villages were dependent on a mix of crop and livestock for production and had very low levels of literacy (14.6%), sanitation (23.8% open defecation), and dietary diversity, and high rates of malnutrition (wasting 10.8%, stunting 21.6%) (10).

## Study design

Given that the study presented here builds on the *Un Oeuf* study, a brief overview of that study is presented here. The *Un Oeuf* study had three research arms—(1) a Full Intervention group, whose child participants were gifted chickens by a community champion at the onset of the project and maternal participants received monthly Integrated Nutrition and Agriculture (INA) trainings for 10 months; (2) a Partial Intervention group, whose participants received the same monthly INA trainings as the Full Intervention group but no chickens until the end of the study, at which time they received two chickens; (3) and a Control group, whose participants received no trainings, but, like the Partial Intervention group, received two chickens upon completion of the study (10). These research arms are preserved for the study presented here. Following the completion of *Un Oeuf*, a closing ceremony recognized the efforts of the participating women and women from the Full Intervention Group educated and trained women in the Control group so that they would benefit from INA training that focused on the importance of feeding your child an egg a day.

This study engages a mixed-methods research approach, analyzing qualitative data alongside existing and new quantitative data. The study examines data from 247 surveys conducted during a follow-up study and analyzes qualitative data collected at endline and follow-up with mothers in 9 of the 18 participating villages (see full *Un Oeuf* sampling strategy and previously published findings (10, 11).

## Quantitative sample, data collection, and analysis

Questions from the *Un Oeuf* study household survey were used for quantitative data collection at follow-up. These included child egg consumption (prevalence and number); poultry production (chicken ownership and number); and mothers' decision-making. To measure egg consumption mothers were asked to report on foods that the child had eaten in the past 7 days and, when eggs were indicated, additional probes quantified the number of eggs. Household poultry ownership was reported as yes/no, and if yes, how many. Participants were asked four questions about household decision-making—*who decides* (1) what foods to feed the children, (2) which foods to purchase, (3) how food is portioned, and (4) what to do with household eggs? Household decision-making variables were coded as binary "self" or "other." Data were managed and analyzed using IBM Statistical Packaging for the Social Sciences (SPSS), and descriptive statistics from follow-up are presented in Table 1.

## Qualitative sample, data collection, limitations, and analysis

A stratified purposive sampling frame was used to select nine of the 18 villages for qualitative data collection. The average number of eggs consumed by the targeted child at midline of the *Un Oeuf* study was used to stratify villages into three groups—low, medium, and high egg consumption. The village with the lowest, average, and highest egg consumption rates at midline (n=3) in each research arm (Full, Partial, and Control; for a total of nine villages) were selected for participation in qualitative data collection.

Qualitative data were collected through FGDs conducted at two time periods: May 2019, immediately following the endline survey of, and August 2019, after follow-up surveys. The FGDs were facilitated using a set of open-ended questions which aimed to identify and explore facilitating factors, barriers, household dynamics, and community-level perceptions relevant to behavior change. Focus group discussions consisted of seven open-ended questions; the guide is included in Supplementary material to this article. Given the difference in the nature of their experience in the study, language in the open-ended questions varied slightly across research arms. Focus groups were administered using a team of three Burkinabe researchers, two of whom were heavily involved with project implementation and quantitative data collection and a local translator with community rapport. The FGDs were conducted using one researcher to facilitate the FGDs in the native language of Moré, whilst the other two researchers simultaneously took separate notes. Despite training and appropriate efforts by

TABLE 1 Egg consumption, household chicken ownership, and household decision-making (HHDM) at the three-month follow-up of the *Un Oeuf* study population by research arm.

| Follow-up summary statistics | | | | |
|---|---|---|---|---|
| | Control $n = 84$ (%) | Partial $n = 85$ (%) | Full $n = 78$ (%) | Total $n = 247$ (%) |
| Egg consumption* | 70 (83.3) | 75 (88.2) | 78 (100) | 223 (90) |
| Mean | 2.9 | 2.6 | 5.74 | 3.70 (2.19) |
| Mode | 3 | 2 | 7 | 2 |
| Range | 7 | 6 | 9 | 10 |
| **HH chicken ownership**† | 84 (100) | 85 (100) | 78 (100) | 246 (100) |
| Mean | 5.12 | 6.18 | 8.77 | 6.64 (3.31) |
| Mode | 4 | 6 | 6 | 6 |
| Range | 13 | 11 | 27 | 28 |
| HHDM | | | | |
| Foods for children | | | | |
| Self | 84 (100) | 85 (100) | 78 (100) | 247 (100) |
| Other | - | - | - | 0 (0) |
| Foods purchased | | | | |
| Self | - | - | 2 (2.6) | 2 (0.8) |
| Other | 84 (100) | 85 (100) | 76 (97.4) | 245 (99.2) |
| Food portions | | | | |
| Self | 84 (100) | 84 (98.8) | 79 (100) | 245 (99.2) |
| Other | - | 1 (1.2) | - | 2 (0.8) |
| Household eggs | | | | |
| Self | 55 (65.5) | 55 (64.7) | 51 (65.4) | 161 (65.2) |
| Other | 29 (34.5) | 30 (35.3) | 27 (34.6) | 86 (34.8) |

*The number (percent) of children reported to have eaten eggs in the past week.
†The number of households that own one or more chickens, followed by the mean, mode, and range of the number of chickens owned.

data collectors, transcripts and field notes indicate that women engaged in the FGDs more like a group interview, where not all women engaged equally in discussion. Following the collection of data, all notes were compiled and checked against the audio recording to ensure a complete capture of each discussion. Data were then translated into a master set of English transcripts for all nine villages.

The FGD transcripts were independently manually coded by two researchers at a US-based university, using thematic analysis to capture salient themes (27). Themes were coded based on patterns that manifested deductively and inductively between the two reviewers. Themes that were inductive were informed by USAID's cross-cutting themes that were considered priorities within the scope of this project. In order to ensure trustworthiness of the data, inter-rater reliability and consensus were established by comparing and negotiating themes independently derived between the two researchers (28). The final list of themes and sub-themes is presented in Table 2. No qualitative research software was used for analysis.

## Results

The results presented in this analysis consist of quantitative and qualitative data collected at the end of the study (FGD) and at a 3-month follow-up (FGD and follow-up surveys). Quantitative research-arm-level findings from baseline and endline of the *Un Oeuf* study have been included in Supplemental material to facilitate comparison with the follow-up data presented below.

### Household survey

At 3-month follow-up, following the end of the *Un Oeuf* study, 247 participants mothers were surveyed. The results for

TABLE 2 Taxonomy of themes and subthemes from focus group discussions with the *Un Oeuf* study participants by research arm.

| Theme | Subtheme | Content by research arm | | |
|---|---|---|---|---|
| | | Control | Partial intervention | Full intervention |
| **Facilitating factors**<br>*A facilitating factor is anything that helped facilitate feeding eggs to the enrolled child* | Household chicken ownership | "[The project] increasing the hens."<br>"We received chickens and now we are able to feed our children eggs." | "[U]sing our hens' eggs for our children. What would have helped us [more] is the donation of hens at the beginning of training."<br>"The increase [to] our bird stock [from the project]." "We [bought] hens for our children. | "Chicken donations help women to feed their children eggs." |
| | Education | "…[A]sking questions each month changed our behavior toward feeding our child eggs (or other foods)." | "The messages from the trainings that will stay with us are: a child an egg daily; handwashing prevents the spread of diseases." | "…[A] child an egg daily, we must clean very well our house and henhouse."<br>"We learned how to take care of our hens, household, and child." |
| **Facilitating factors**<br>*A facilitating factor is anything that helped facilitate feeding eggs to the enrolled child* | Spousal Support | "[The spouses support us] by giving us permission to participate in the survey."<br>"My husband built a hen house for the hens [donated by the project] and paid [for] a rooster to add to the hens…" | "Our spouse's hen donation [help feed our children]."<br>"My husband has built a chicken house for my chickens."<br>"My husband often calls the vet to vaccinate our chickens." | "[Our husbands] encourage us to participate in the training."<br>"Our husbands often help us in breeding chickens." |
| **Barriers**<br>*A barrier is anything that prevents the feeding of eggs to the enrolled child* | Egg production | "The low number of [egg] laying hens."<br>"If our hens don't eat well, they cannot lay eggs." | "When hens brood and we do not have money to buy eggs."<br>"We [do] not have many hens."<br>"The [two] hens do not lay enough eggs." | "If the hens are sick and do not lay."<br>"If the hens are not well fed, they will not lay [eggs]."<br>"…[A]t the beginning the hens were [too] young." |
| | Cultural taboos[a] | "Barriers keep us from feeding our child an egg daily, the social and cultural barrier [that] a child must not eat eggs." | "At the beginning we did not feed children eggs because of traditional barriers, but since we received the training, we are feeding our children eggs." | |
| **Barriers**<br>*A barrier is anything that prevents the feeding of eggs to the enrolled child* | Animal health | "When hens do not have a hen house; they lay eggs where they want."<br>"[H]ens lack food."<br>"The non-existence of a hen house for hens…if it rains, I am obliged to put my hens and their chicks in our house…"<br>"Avian pathologies can decimate hens."<br>"[M]aintenance and follow-up of the hens." | "Unfortunately, I only have one hen now, the other hens are dead, so I can't get eggs for the child. I have to buy eggs for my child."<br>"When there is no local veterinarian to vaccinate our hens… they will become sick." | "If we don't have medicines to give to the hens when they are sick."<br>"If we don't have hen houses."<br>"[I]f you do not have hen houses, it will be difficult to take care of poultry."<br>"How are we going to take care of our poultry without you?" |
| **Motivational factors**<br>*A motivational factor is anything that motivated and inspired the mothers to start or continue feeding eggs to the enrolled child* | Health of child | "Our child is very healthy, compared to other children, his weight is normal."[b]<br>"…[C]hicken eggs improve his growth and intelligence." | "All my children were malnourished and since I started giving eggs to this child, he is doing well. [He] is not malnourished [like] his other siblings." | "[I] can see an impact of the project on our child. [He is] very healthy, are in top form. [He is] are growing well compared to other children his age who are not enrolled." |

*(Continued)*


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TABLE 2 (Continued)

| Theme | Subtheme | Content by research arm | | |
|---|---|---|---|---|
| | | Control | Partial intervention | Full intervention |
| **Motivational factors** *A motivational factor is anything that motivated and inspired the mothers to start or continue feeding eggs to the enrolled child* | Health of child | "... [E]ating eggs helps children to avoid some diseases. | "The fact that we see our children are healthy motivates [us]." | "... We have more respect for our community leader, to know they turned [our] attention to our children's nutrition shows we must take care very well of children's hens." |
| | Time | "There is the decrease of breastfeeding of children thanks to the eggs." "There is Partial release of mothers and increase mothers' household time." "There is the saving of time (and money) by mothers thanks to the good health of the children." | "There was a change, the children suckle less and are healthy. [W]e are also healthy." | "As a mother, [I am] satisfied there is a decrease in child breastfeeding through egg consumption." "When a child eats an egg, he suckles less and gives a lot of free time to the mother to do her activities." |
| **Motivational factors** *A motivational factor is anything that motivated and inspired the mothers to start or continue feeding eggs to the enrolled child* | Financial independence | "We will have profits because we [now] have chicks [from the project donation] and they will become chickens we will sell some of them to support certain needs." | "As mothers, we are happy. It will benefit [us] if we have many chickens; we can sometimes sell some chickens to support our child's needs or our needs." | "I am happy to have chickens. I can often sell some chickens to pay [for] my child's clothes." "We learned a lot about poultry breeding ... now we can take care [of] or breed poultry ourselves." "I sold some chickens to pay for a small ruminant for my child." "As a mother, we see a difference [between] other mothers who did not receive the chickens. The mother who received the chickens is financially independent..." |
| **Knowledge-sharing** *Mothers within the full and Partial Intervention arms were sharing their knowledge, whilst mothers in the Control were eagerly accepting of it when shared* | Community | "We heard about the program from one of our family members." "... [T]he project has changed how we interact within our household and our community, because we tell other women in our community what we learned about children's nutrition during the survey." "... [O]ther women in the village put into practice the advice and some would like to participate in the program." | "[We shared] the benefits of egg consumption, child hygiene, and sanitation." "When we go home after training sessions, we share what we learned with our neighbors." "[We shared this information] because it will help other women to take care of their children." | "... [W]e use the flipbooks to share information with women outside our community." "We share this information with women outside in our community (village) who are participating in this project." "[This] benefited us, so we want the same thing for [other mothers]". |
| | Household | "I shared this with my husband's second wife." "In our household, the project has changed our behaviors around health and hygiene of [our] children." | "There is the involvement of household members in poultry monitoring." [We share information] with our husband's second wife." | "Behaviors [that] have changed in our household are our children's hygiene and nutrition, [and] poultry's hygiene." |
| **Sustainability** *Sustainability within the population is what the participants planned to do to maintain egg consumption within their households* | Behavior Reinforcement | "We will take care of the chickens to always have eggs." "We will remind each other what we must do." "We will take care of the chickens to have eggs at all times." | "We will use our flipbooks. Our flipbooks contain information that helps us to put into practice what we learned during training sessions." "We have our flipbooks that will help us to continue [to] remember everything we learned during our training and put it into practice | "We will use our flipbooks to remember." "We will vaccinate our chickens. [T]here are people in the villages who can vaccinate our chickens." "[I] will always apply the creed: 'a child an egg a day,' even if [I] give birth to another child." |

[a]There were no reports of cultural taboos being a barrier within the Full Research Arm.

[b]It is important to note that mothers in the Control Group mentioned the health of their children in relativity to no other children that were enrolled in the study. This shows the skewed perspective of health that can occur when a village has low dietary diversity and faces food insecurity.

egg consumption, household chicken ownership, and household decision-making can be found in Table 1. Follow-up survey data indicate that egg consumption expanded through follow-up; 223 children (90%) consumed eggs the week prior to data collection, with an average consumption of 3.7 eggs per week. In the Full Intervention group, all children (100%) were consuming eggs at follow-up, compared to 83.3 and 88.2% in the Control and Partial Intervention groups, respectively. In all three research arms, all households reported owning chickens (100%) at follow-up, with an average number of chickens of 6.64 (range 2–30). The average number of chickens remained higher in the Full Intervention group than the Partial Intervention group (8.8 compared to 6.2), both of which were higher than the Control group (5.1). Very little difference was observed in decision-making at follow-up: 100% of all mothers reported making household decisions about what foods are fed to children; and 99% (all but one mother) reported being the decision maker about how foods are portioned. Two women (0.8%) reported making decisions about what foods to purchase; importantly, both women were in the Full Intervention arm. Finally, 161 women (65%) in the study population reported being decision maker about what is done with the household eggs; distribution was comparable across all three groups (65.4, 65.5, and 64.7% in the Full, Control, and Partial Intervention groups, respectively).

## Focus group discussion

The FGDs yielded six prominent themes—facilitating factors, barriers, motivational factors, livelihood, knowledge-sharing, and sustainability (Table 2). Each theme has been operationalized with a definition provided in Table 2.

## Facilitating factors

Within the theme of facilitating factors, three subthemes were identified—household chicken ownership, education, and spousal support. At follow-up, all research arms reported that an increase in the household chicken ownership facilitated the mothers to feed the enrolled child eggs. While the delivery of education varied across research arms, there was consensus that increasing knowledge (and awareness) was a key facilitating factor in the behavior change of feeding eggs to children—including the Control group which only received educational materials in the form of a “flipbook” when they received chickens for the household at the end of project ceremony. Unintended education for the Control group came in the form of the household survey, which brought attention to and started conversations on feeding eggs (and other nutritionally rich foods) to children. For the Partial and Full groups, the education was much more formalized through the implementation of INA trainings that were attended each month, as well as educational materials (i.e., flipbooks) that were given to mothers. That mothers kept and owned their own flipbook, which facilitated their ability to refer to the flipbooks at any time, was a key component in the education on behavior change toward feeding children eggs.

There was a consensus in all villages that the support of husbands was integral to facilitation of egg consumption, including the relinquishing of household decision-making over what the child(ren) consumed. Women reported that their husbands were supportive and encouraging of the women’s involvement in the study, and that they helped facilitate egg consumption by giving eggs or hens from the household flocks, purchasing chickens so eggs would be available, building hen houses, and helping in the care of the chickens.

## Barriers

The theme of barriers consisted of the subthemes of egg production, cultural taboos, and animal health. Importantly, women in the Full Intervention arm reported that any barrier present and initially limiting egg consumption were overcome by the study design.

The lack of egg production was a barrier for all research arms. As expected, women reported that hens do not lay eggs when sick. Additionally, at the beginning of the intervention, hens in the Full Intervention arm were too young to lay eggs; therefore, women in the full group experienced a lack of egg production due to having young hen flocks. Women reported that when hens brood or laid no eggs, it was a burden to need to purchase eggs for the child.

Within this region of Burkina Faso, a cultural taboo surrounding the consumption of eggs by children—particularly young girls—was identified as a barrier to egg consumption by participants. This taboo was identified during formative research and therefor included and addressed in early training sessions of the mothers. With support of community champions, acting as advocates for egg consumption, once women understood that consuming eggs was beneficial to a child’s health, this taboo no longer limited women’s willingness to feed children eggs in the Partial and Control research arms.

Another consistent barrier across research arms was the health of the animals. Women expressed that when they lack the ability to properly feed their hens, the hens fall ill, with consequences on egg production and on flock size. Additionally, the financial barriers to construct a hen house, which also then implied feeding the hens, left the birds subject to predators and weather. Limited vaccine availability was also identified as a cause for poor hen health.

## Motivational factors

Child health and time-gain of the mothers were the two subthemes deduced from the theme of motivational factors.

The health of the child was the overarching and most-reported motivational factor across all groups. Mothers all agreed that the most motivating reason behind their behavior change of feeding the children eggs was the improvement in the children's nutrition, growth, and overall health.

Mothers agreed that with the addition of eggs in the children's diets, the children demanded to suckle less; therefore, reducing the time-demand on the mother. Because of this release from breastfeeding, mothers reported this increase in available time as a motivational factor because they were able to better care for themselves and their households.

### Livelihood

Within livelihood, the subtheme of financial independence arose, strictly surrounding the benefits yielded from poultry farming. Mothers agreed that there was a newfound sense of financial independence due to the increase that poultry production brought them. Particularly in the full group, women reported being able to purchase clothing for her children, pay for school fees, as well as purchase small ruminants and other foods to increase the dietary diversity of the children.

### Knowledge-sharing

Knowledge-sharing was shown within two subthemes—community and household. Knowledge-sharing within the community, both at the village-level and broader department-level, was witnessed across all research arms. Once mothers had knowledge to share, they shared it. Women explained they did this so that other women would have healthier children. This knowledge was shared for the good of the greater community. Knowledge-sharing within the households took place between the mothers who were enrolled in the study and their husband and co-wives. This knowledge was exchanged for the betterment of the entire family unit.

### Sustainability

Sustainability in the sense of behavior reinforcement was expressed across all three research arms. Since behavior change is an iterative process, behavior reinforcement is key in sustaining behavior change. Women explained their desire to always properly care for their chickens to ensure their children always had eggs. Additionally, women stated that they would continue to use and share the information in the flipbooks, provided by the project. The flipbooks contained picture-based information about poultry management, child diet, and IYC feeding practices, all elements that the women saw as reminders of what is needed to improve child health through poultry production. Women appreciated, shared, and valued the flipbooks.

## Discussion

This study was launched in order to identify factors that allowed for significant increase in IYC egg consumption during the *Un Oeuf* study and to assess the sustainability and scalability of the approach. As previously published, at endline of the intervention, 100% of children in the Full Intervention arm were consuming eggs (11). The results of the qualitative data revealed that women in the Full Intervention group were able to overcome any barriers that presented at the beginning of the study, with attribution to intervention design elements, including training (increased education and understanding of eggs' nutritional value) and livestock assets (increased flock size), both embedded in a culturally sensitive approach (the support and advise of a trusted community champion during the gifting ceremony). Important motivational factors identified by the women included the observation of improvements in child health and increased time availability. Observation of visible improvements in child health acted as a natural reinforcer for mothers feeding their children eggs. Natural reinforcers, a type of positive reinforcement (improved health) that occurs because of a behavior change (egg consumption), are well-documented in behavioral psychology as instrumental to sustained behavior change (29, 30). No previous studies were identified that documented mothers' observation of IYC growth as motivational for adherence a nutritional intervention in a LMIC. The alleviation of time poverty that afflicts mothers (31, 32), allowed the mothers to better care for all members of their households, including themselves, a finding that supports previous research (33, 34). Mothers also reported new levels of financial independence, a critical pathway for improving maternal and child health and nutrition (25, 35, 36). While facilitating factors such as education about the nutritional value of eggs and ongoing spousal support allowed for continued IYC egg consumption at follow-up, egg availability emerged as an important constraint, limiting the frequency of IYC egg consumption, even among women where motivation remained high.

Data presented here indicate that the high prevalence of IYC egg consumption in the Full Intervention group persisted at 3-month follow up (100%). However, the number of eggs consumed per week dropped from 6.3 to 5.7 from endline to follow-up. This drop was matched by a precipitous drop in chicken ownership: while all households in the Full Intervention arm had chickens at endline and at follow-up (no change, 100% at each observation), the mean number of chickens dropped from 19 chickens to 9 by follow-up. These data reflect that which was described in FGD, that women in the Full Intervention group were highly motivated at endline and at a 3-month follow-up to feed their child an egg a day, but they began to face some constraints related to poultry numbers by follow-up.

Across research arms, mothers reported the number of chickens and associated number of eggs to be one of the most important determinants to their ability to feed their child an egg. This supports findings from previous research, which finds important associations between animal ownership and ASF consumption, most often milk, poultry, and eggs (25, 37–40). Women in the Full Intervention group credited the influx of (four) chickens into their household as being life changing for their livelihoods, while mothers in the Partial Intervention and Control groups stated that the project's donation of two hens at endline was instrumental in creating behavior change, as well as freeing up household income previously spent on purchase of eggs. Focus group discussions indicated that animal health, including vaccination, housing, and other key management strategies, were instrumental in increasing poultry production (number of chickens) and productivity (number of eggs). The INA trainings that addressed these issues were highly valued by participants seen as contributing to the increased flock numbers and egg consumption at endline. This was supported by some literature (41), but other interventions that have taken similar tacks did not succeed in increasing poultry production (42). Limitations, such as the inability of the project to assist women in the *Un Oeuf* study with construction of chicken houses, were also identified.

Despite the prevalence of child egg consumption increasing between endline and follow-up in the Partial Intervention and Control arms to percentages much closer to the Full Intervention arm, the mean number of eggs consumed per week by children in the Partial Intervention and Control groups remained lower (2.6 and 2.9, respectively) than in the Full Intervention group (5.7). In conjunction with qualitative findings and the observed drop in poultry numbers and egg consumption in the Full Intervention group, this observation calls into question a possible threshold of poultry numbers sufficient for egg consumption, as the Full Intervention arm had almost nine chickens at follow-up, which was higher than the Partial (6) or Control (5) group. This supports previous research which found the type and number of livestock to be important determinants of any improvements in child diet or growth (37). In addition, the excess poultry production in the Full Intervention group at endline allowed mothers a sustainable means of livelihood – something that could be sold to support the purchase of other household needs, including medicines, other types of foods that increased the household dietary diversity, clothing, and school fees for children. This reinforces livelihood support interventions that focus on utility and economic improvement to address health-related issues (35, 43).

The second objective of the study was to assess the sustainability and scalability of the intervention. As indicated above, the motivation to feed children an egg a day and a relatively strong ability to do so remained high in the Full Intervention group 3 months after the project ended, indicating that the original intervention was sustainable, at least in the short term. The question of scalability—can this intervention be taken elsewhere and replicated—appears to hold strong potential. The concept of gifting chickens to children by a community champion utilized in this project was piloted by Omer et al. in Ethiopia, where comparably strong results were found (26). In the *Un Oeuf* study, the behavior change strategy leveraged this culturally sensitive approach and focused on increasing egg availability, through poultry ownership and education about poultry production, and increasing motivation and decision-making to feed the child eggs, through education about child nutrition. A policy roundtable, held in January 2020 following the end of the project, reinforced the potential of chicken-gifting by community champions (44). Using the socio-ecological model (SEM) the study design actively engaged multiple levels of support, including household (spousal support) and community level (community champions), which proved instrumental (45, 46). Husbands were invited and encouraged to attend the INA trainings with their enrolled wives, and some contributed chickens, either during the initial ceremony with the village leaders or subsequently on their own. This support from husbands was identified as an important facilitating factor to women continuing to feed children eggs over time. This supports existing literature on the important role of men/fathers in IYC nutrition programs (47–50), as well as literature on the multilevel factors that affect child nutritional practices (51). Though future research is needed to confirm how many chickens are required to produce enough eggs to support a standard of one egg per child each day, the *Un Oeuf* approach appears to be scalable to other smallholder farming communities to improve child diets through increased egg consumption. Such an approach would utilize the SEM to design a culturally tailored intervention in which community champions gift poultry to IYC, and mothers participate in tailored monthly INA trainings (in which fathers are welcome) that reinforce best practices in poultry production and knowledge and practices that support leveraging the nutritional value of eggs for optimal child growth.

## Conclusion

The Full Intervention tested in *Un Oeuf* had a transformative impact on the lives of mothers and IYC by altering the ways in which mothers breed chickens, feed their children, and care for their households. At the onset of the study, chicken eggs were sparsely eaten by children, largely due to poverty, lack of knowledge about nutritional value of eggs, and cultural norms that described eggs as part of a chicken's lifecycle rather than a food source (11). The intervention overcame these barriers and behavior change was sustained 3 months following the study. The *Un Oeuf* study contributes to literature which underscores the potential of using cultural pathways to trigger and sustain nutritional behavior change, even when it challenges cultural norms. Nutrition-sensitive, livestock-based interventions in LMIC may consider tailoring the *Un Oeuf* approach to catalyze and sustain egg consumption in children and other undernourished populations.

## 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://dataverse.harvard.edu/dataverse/livestock-lab-burkina-egg-consumption.

## Ethics statement

The studies involving human participants were reviewed and approved by University of Florida Institutional Review Board Burkina Faso Ethical Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## Author contributions

EM contributed to securing funding, data collection, data management, analysis, and primary writing. EW contributed to analysis and primary writing. HS contributed as co-PI to securing funding, study design, implementation, data management, and primary writing. AW contributed as co-PI to securing funding, study design, and implementation. SM contributed as principal investigator to the project to secure funding, study design, implementation, data management, and primary writing. 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.2022.1096256/full#supplementary-material

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# Impact of nutritional diet therapy on premenstrual syndrome

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Premenstrual syndrome (PMS) is one of the most common disorders faced by women of reproductive age. More than 200 symptoms of varying severity associated with PMS have been identified. Because of the broad spectrum of action of PMS and its impact on quality of life, symptom relief is the main challenge of treating PMS and premenstrual dysphoric disorder (PMDD). The review aims to analyze and identify the potential impact of dietary and nutritional therapies on PMS and, respectively, for its better management. The study was conducted by accessing Internet databases such as PubMed, ScienceDirect, and Scopus and using relevant keywords such as PMS, symptoms, dietary patterns (DPs), macro and micronutrients, and supplements. The results showed that diet is an essential modulating factor in reducing and managing PMS symptoms. But research on the actual effect of foods and nutrients on PMS is sparse, sporadic, and studied with insufficient scientific rigor. No correlations were identified between the consumption of macronutrients and PMS: protein, fat, carbohydrates, and fiber, but the effectiveness of micronutrients, especially calcium, magnesium, vitamin D, B vitamins, and herbal supplements, was demonstrated. Researchers remain unanimous that the evidence is insufficient and limited to support their use as an effective treatment. Nevertheless, the results could contribute to providing quality information to help women and girls make evidence-based decisions regarding premenstrual health and the adoption of dietary and nutritional therapies.

KEYWORDS

**premenstrual syndrome, food patterns, nutrients, supplements, menstrual cycle, wellbeing**

## 1. Introduction

Women's wellbeing is one of the health's main goals and is an increasingly good tool for determining the functional impact of some diseases. It also indicates social and economic development and quality of life. The most common problems faced by women, mentioned in the literature, are related to the menstrual cycle (1). Premenstrual syndrome (PMS) is one of the most widespread disorders in reproductive age, negatively impacting women's emotions, and performance (2). Although the first symptoms, similar to PMS, were described as early as Hippocrates, the diagnostic criteria were specified more recently. Mainly due to the heterogeneity of menstrual symptoms, definitions have varied substantially over the years, evolving from "menstrual moods," "premenstrual tension," to "PMS" (3–6). In the late 20s and mid-50s, PMS comes more frequently to the attention of researchers (7) (**Figure 1**).

PMS is a clinical condition that occurs during the luteal menstrual cycle, that is, during the last 14 days of the menstrual cycle (from ovulation to the onset of menstruation). It is characterized by the cyclic presence of recurrent affective, physical, and behavioral symptoms, which disappear spontaneously within 4 days from the beginning of menstruation and do not recur until at least the day of the cycle (8–11).

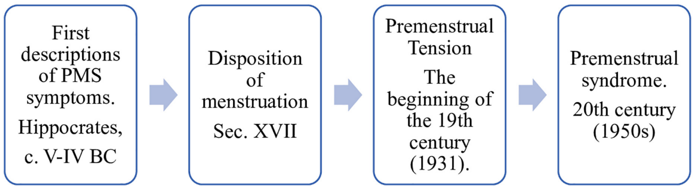


FIGURE 1
Evolution of the nomenclature for menstrual disorders.

Late luteal dysphoric disorder (LLDD), also known as premenstrual dysphoric disorder (PMDD), is the most severe form of PMS. It is considered a medical condition, severely disrupting women's quality of life, often causing them to seek drug treatment. Currently, PMDD is listed in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) as a separate entity under depressive disorders (6, 12).

Due to the poor understanding of the mechanisms underlying PMS, the exact etiology of these premenstrual disorders remains unclear and effective treatments are limited (13). The most well-known hypotheses concerning PMS are associated with hormonal fluctuations, following ovulation, diets with nutritional deficiencies (especially in vitamin B6, magnesium, and calcium), family medical history, which includes depression or anxiety, etc. Symptoms may begin in the early, mid, or late luteal phase and are not associated with defined concentrations of any specific gonadal or non-gonadal hormone (14). Other arguments focus on abnormal serotonergic activity, progesterone, and gamma-aminobutyric acid (GABA) neurotransmitter aberrations (15) and the presumed role of circulating gonadal steroids in the development of symptoms (16, 17). Dibaz and Aksan (16) argue that sex steroids and neurotransmitters play a central role in the etiology of PMS. There is evidence that PMS is twice as pronounced in women with a normal body mass index (BMI) compared to women with a BMI $\geq$ 25 kg/m$^2$ (18).

Women with PMS experience affective or somatic symptoms that cause severe social or occupational dysfunction (14). The range of symptoms associated with PMS is extensive, varying in severity, differentiating from one individual to another, and extending across a range of medical specialties: from gynecological to psychiatric, affecting all aspects of life (11). Over 200 signs and symptoms of PMS have been identified (19). Up to 98% of women report at least one physical and mental symptom before the onset of their menstrual cycle. About 30–40% of women say PMS symptoms that involve drug treatment, and 3–8% of women suffer from PMDD that meets strict DSM-IV criteria (7, 8, 15, 16, 20–24). The most common symptoms are shown in Table 1.

The persistence of symptoms tends to fluctuate, with prevalence influenced by cultural and geographic characteristics. For example, France has the lowest PMS rate (12%), and Iran has the highest rate (98%). PMS is not associated with age, educational level, or financial means (8). Depending on the severity of symptoms, PMS can lead to decreased quality of life, reduced occupational productivity, increased dependence on specialized medical care, and interference with interpersonal relationships and daily activities. In addition, PMS may increase the risk of hypertension, negatively impact athletes' performance and daily activities, and is significantly associated with reduced academic performance (25). The diagnosis of PMS consists of identifying the timing of symptoms about menstruation, the significant change between the severity of post- and premenstrual symptoms, and the significant severity of clinical symptoms (2, 26).

TABLE 1 The most common symptoms of premenstrual syndrome (PMS).

| Physical symptoms | Psychological and behavioral symptoms |
|---|---|
| Weight gain | Insomnia/Drowsiness |
| Edema | Change in appetite: increased appetite |
| Breast tenderness and swelling | Anxiety and tension |
| Stomach problems | Decreased libido |
| Back pain | Depressed mood |
| Muscle pain | Changes of disposition |
| Joint pains | State of fatigue |
| Headaches | Anger |
| Dizziness | Irritability |
| Sweating | Weeping |
| Acne or other skin problems | Restlessness |
| Constipation or diarrhea | Confusion |
| Bloating and flatulence | Concentration and memory problems |
| Cramps | Loss of confidence |
| Low tolerance to noise and light | Social isolation |

## 1.1. Treatment

Better definitions and research based on strict inclusion-exclusion criteria have allowed the development of effective treatments adapted to the severity of lifestyle disruption and specific individual symptom totals (27). However, due to the broad spectrum of action of PMS and its impact on quality of life, symptom relief is the main challenge of treating PMS and PMDD (28).

This review aims to analyze and identify the potential impact of dietary and nutritional therapies on PMS and, respectively, for its better management.

The study was conducted by accessing Internet databases such as PubMed, ScienceDirect, and Scopus and using relevant keywords such as PMS, symptoms, dietary patterns (DPs), macro and micronutrients, and supplements.

# 2. Impact of dietary patterns and macronutrients on PMS

Although people who experience severe PMS symptoms often require medical intervention, most women repress them without diagnosis or management. To date, no treatment is universally recognized as effective, and many women seeking relief often turn to therapeutic approaches outside of conventional medicine (29, 30).

Diet appears to be an essential modulating factor in reducing and managing some of the symptoms of PMS. But the actual effect that foods and nutrients have on women with menstrual disorders is not studied with enough scientific rigor (2, 31). It is recommended to follow a healthy food model, in which fresh, unprocessed foods predominate and avoid those rich in carbohydrates or refined fats, salt, alcohol, and stimulant drinks.

Following a healthy diet and managing stress are important factors in preventing and managing PMS (32).

In a study that looked at the impact of three DPs: traditional DP, high in eggs, tomato sauce, fruit, and red meat; healthy DP, rich in dried fruits, spices, and nuts and Western DP, characterized by high consumption of fast food, carbonated drinks, and processed meat. Western DPs were positively associated with PMS, while healthy and traditional nutritional habits had an inverse correlation (33). Research has shown that short-term intermittent fasting can lead to more excellent parasympathetic activity and lower luteal cortisol levels in young women. These results indicate the possibility of producing an anti-stress effect in the luteal phase, which would reduce menstrual symptoms (34, 35).

It has been suggested that caloric intake, as well as preferential carbohydrate selection, during the premenstrual period is more significant in women with PMS, who are considered to be more sensitive to cyclical hormonal or neurotransmitter fluctuations (36). The improvement in mood after carbohydrate ingestion is explained by the increase in serotonin associated with tryptophan, ameliorating a potentially functional deficiency of serotonin in the brain and thus serving as self-medication. At the same time, a diet with excess sugars, especially simple fats, fried foods, coffee, and alcohol, correlates positively with the development of PMS. In order to reduce PMS symptoms, the authors recommend a diet rich in vegetables, fruits, and healthy fiber (23, 37–39).

Other research, which studied the impact of macronutrient intake on PMS, reported that no correlation was found between the consumption of protein, fat, carbohydrates, fiber, and PMS. But it is suggested that maltose might be associated with PMS (37, 40), and high intake of stearic acid may be associated with a lower risk of developing PMS. Further prospective research is needed to confirm this finding (23).

Tests were carried out on subjects who followed diets in which 40% of energy came from fat, alternated with periods of the regime with only 20% of energy from fat. The subjects were randomized into two categories: one category that had a ratio of polyunsaturated and saturated fatty acids of 1.0 and another group–with a ratio of 0.3. There were no significant differences in self-reported menstrual symptoms between the two groups (polyunsaturated/saturated), but significant decreases in symptoms associated with water retention were reported (41). Total fat intakes (saturated and monounsaturated) were significantly correlated with pain symptoms (42).

## 3. Impact of micronutrients and herbal supplements

Zinc is known to have multiple beneficial effects, including anti-inflammatory, antioxidant, and antidepressant actions. Overall, zinc supplementation for 12 weeks among women with PMS had a beneficial impact on physical and psychological symptoms, total antioxidant capacity, and brain-derived neurotrophic factor. However, data on the effects of zinc supplementation on biomarkers of inflammation, oxidative stress, and antidepressant impact among young women with PMS are scarce (43).

Several studies have shown that subjects with PMS have lower serum calcium levels, and calcium supplementation could significantly improve the incidence of PMS and its associated symptoms (44–47). However, further clinical studies are needed to establish a firm link between calcium and PMS (48). Other research has justified the approach of a high intake of calcium associated with vitamin D in reducing PMS symptoms, including lowering the risk of osteoporosis and some cancers (49). Calcium and vitamin D supplementation is recommended as an inexpensive, low-risk, acceptable, and accessible approach to eliminate or reduce symptoms (50). Still, it is not known whether these nutrients can prevent the initial development of PMS (49). Various studies show the importance of vitamin D in female reproduction, probably due to its effects on calcium homeostasis, cyclic sex steroid hormone fluctuations, or neurotransmitter function (51–54). It also helps reduce dysmenorrhea, inflammation, and antioxidant markers in women with PMS and vitamin D deficiency (55, 56). In adolescents, vitamin D therapy is associated with improvements in PMS-related quality of life and mood disorders (57). There is also research showing that vitamin D supplementation for 12 weeks had no significant impact on other PMS symptoms (58).

Magnesium supplementation is considered effective in preventing dysmenorrhea, PMS, and menstrual migraine (59). A combination of magnesium with vitamin B6 can effectively reduce premenstrual stress, and vitamin B6 can effectively reduce anxiety in older women (60).

Thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), folic acid (B9), and cobalamin (B12) are indispensable vitamins in the synthesis of neurotransmitters potentially involved in the pathophysiology of PMS (61). Research on the impact of dietary intake of niacin, pyridoxine, folate, and cobalamin on PMS has not shown significant associations. Intake of B vitamins from supplements was not associated with a lower risk of PMS. But, a significantly lower risk of PMS was observed in women with a high intake of thiamin and riboflavin from food sources (61).

A comparative study, with reference to the effectiveness between vitamin B6 and broad-spectrum micronutrient formulas (which included minerals and vitamins) on PMS showed that both treatments provided similar benefits: the micronutrient formulas had a more significant effect on the quality of life, as well as a potential clinical use for PMDD. However, vitamin B6 therapy appears to be as effective as broad-spectrum formulas (62). In another study, which looked at the impact of 62 herbs, vitamins, and minerals on PMS, only calcium intake was justified in reducing PMS. The authors argue that further research, with sufficient sample sizes and measuring the effect on individual PMS symptom severity, is needed (30) to support the use of calcium, vitamin D, and vitamin B6 supplements, as well as herbal remedies. Evidence supporting cognitive behavioral therapy is also insufficient (28, 63).

Nor is the effectiveness of dietary supplements sufficiently researched. Although some research claims that neither evening primrose oil nor St. John's wort has any different effect than placebo (29), other research shows the positive impact of evening primrose oil on PMS (32). The potential beneficial effect of curcumin in alleviating the severity of PMS symptoms, possibly mediated by curcumin's neurotransmitter modulation and anti-inflammatory effects, is also noted (64, 65). Jafari et al. (66) highlights the potential impact of garlic in reducing the severity of PMS and its possible use as an alternative therapy in the prophylaxis and treatment of premenstrual disorders.

Limited evidence supports the promotion of a healthy diet, exercise, and vitamin supplementation in reducing PMS, but their advertising is recommended for their apparent health benefits in general. Lifestyle modification and regular exercise may have a more pronounced positive effect in milder cases of PMS (16). At the same time, it is recommended to minimize the intake of salt, caffeine, and tobacco (15, 67–69). A study of students in the United Arab Emirates reported that fruit consumption was associated with a reduced risk of behavioral symptoms, and smoking and consumption of caloric foods (high in fat, sugar, and salt) were identified as risk factors vital for PMS (25).

## 4. Conclusion

- The range of symptoms associated with PMS is extensive, with varying severity, extending to most aspects of women's lives, and requiring knowledge, monitoring, and a personalized approach to diagnosing psychological and physical conditions.
- The best-known hypotheses, which explain the causes of PMS, are associated with hormonal fluctuations and nutritional deficiencies, especially in vitamin B6, magnesium, and calcium.
- To date, no treatment is universally recognized as effective, and many women seeking relief often turn to therapeutic approaches outside of conventional medicine.
- Diet is an essential modulating factor in reducing and managing PMS symptoms. But research on the actual effect of foods and nutrients on PMS is sparse, sporadic, and studied with insufficient scientific rigor.
- The study did not identify correlations between the consumption of macronutrients: proteins, fats, carbohydrates, fibers, and PMS. But it is suggested that maltose might be associated with PMS.
- Some studies have shown the effectiveness of micronutrients, especially calcium, magnesium, vitamin D, B vitamins, and herbal supplements, in reducing PMS. But researchers agree that the evidence is insufficient and limited to support their use as an effective treatment.
- Lifestyle, nutrition, and general health considerations appear to be essential strategies in the reduction or management of menstrual symptoms but are recommended to be promoted more for their apparent health benefits than as conclusive evidence for reducing negative experiences of PMS.
- Awareness of health and nutrition professionals to inform the public about the complexity of factors influencing PMS and the need for training/education regarding self-care practices for PMS management is current and necessary. At the same time, nutrition service providers should approach and adapt dietetic-nutritional therapy in a personalized way to reduce PMS. In addition, to have and provide quality information to help women, including young women/adolescents, make evidence-based decisions about premenstrual health and the adoption of diets, nutrients, or supplements.

## Author contributions

Both 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



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# Health effects of the time-restricted eating in adults with obesity: A systematic review and meta-analysis

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**Background:** The number of people suffering from overweight or obesity has been steadily increasing in recent years. As a new form of diet, the efficacy of time-restricted eating (TRE) remains debatable.

**Objective:** This meta-analysis quantified the effect of TRE on weight change and other physical parameters in obese and overweight adults.

**Methods:** We did a systematic review and meta-analysis of randomized controlled trials (RCTs) comparing the TRE interventions on weight loss and other metabolic parameters by searching PubMed, Embase, and Cochrane Central Register of Controlled Trials to identify eligible trials published from database inception up until 23 August 2022. The risk of bias was assessed using the Revised Cochrane risk-of-bias tool (ROB-2.0). Meta-analysis was performed using Review Manager 5.4.1 software.

**Results:** Nine RCTs with 665 individuals (345 in the TRE group while 320 in the control group) were included. Results indicated that TRE had a greater decrease in body weight (−1.28 kg; 95% CI [−2.05, −0.52], $p$ = 0.001), fat mass (−0.72 kg; 95% CI [−1.40, −0.03], $p$ = 0.04), body mass index (−0.34 kg/m$^2$; 95% CI [−0.64, −0.04], $p$ = 0.03) and diastolic blood pressure (−2.26 mmHg 95% CI [−4.02, −0.50], $p$ = 0.01). However, the meta-analysis demonstrated that there was no significant difference between TRE and the control group in lean mass, systolic blood pressure, waist circumference, fasting glucose, fasting insulin, homeostasis model assessment-insulin resistance (HOMA-IR), total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides. Besides, the duration of the study and daily eating window also had an impact on weight change.

**Conclusion:** TRE was associated with reductions in weight and fat mass and can be a dietary intervention option for adults with obesity. But high-quality trials and longer follow-ups are needed to draw definitive conclusions.

KEYWORDS

**time-restricted eating, obesity, overweight, dietary interventions, meta-analysis**

# 1. Introduction

Obesity has reached epidemic proportions around the world, with approximately 39% of adults classified as overweight and more than 600 million classified as clinically obese by 2020 (1). Considered an epidemic and, consequently, a public health problem, it is not only directly associated with non-communicable diseases and chronic diseases, such as diabetes mellitus, cardiovascular diseases, brain stroke, certain cancers, obstructive sleep apnea, and osteoarthritis, but also has important consequences for disability, emotional wellbeing, and quality of life (2, 3).

Some studies have observed an association between weight loss and improvement in some cardiometabolic markers such as serum triglycerides and cholesterol, free fatty acids, leptinemia, glucose, insulinemia, and blood pressure (4–8). Body weight and fat mass are regulated by many physiological mechanisms, energy imbalance due to increased caloric intake and reduced physical activity is one of the major causes of obesity in adults (9). Lifestyle interventions, including qualitative and quantitative nutritional changes, as well as increased exercise, have been the first line of treatment for obesity and metabolic diseases. However, body weight is regulated by numerous physiological mechanisms, far beyond voluntary food intake, and physical exercise. When a person loses weight the body fights back, with physiological adaptations on both sides of the energy balance equation that try to bring body weight back to its original state (10, 11).

Surrounded by highly palatable and energy-dense processed foods, many people tend to consume more energy than they burn, making it difficult to achieve sustained clinically significant weight loss by long-term calorie restriction (12). Treatment of obesity is multidisciplinary, with lifestyle changes being the first option, including changes in food choices and increased levels of physical activity (13). The investigation of dietary approaches that may promote patient adherence to treatment is a fruitful area of research (14).

As reported in the review of the literature in 2020, intermittent fasting which is a dietary pattern based upon timed periods of fasting, is beneficial in preclinical and clinical studies in a variety of conditions like obesity, diabetes, heart disease, cancer, as well as neurological disorders (15). Given the various options for intermittent fasting, time-restricted eating (TRE) has gained scientific attention in recent years. This approach proposes a fasting period of 8–12 h/day, followed by a period of free eating or eating associated with energy restriction (16). The method has gained popularity because it is a simple and easy weight loss strategy, which may improve adherence rates (17). TRE prevents weight gain in mice with a high-fat, isocaloric diet (18), and reduces body weight and metabolic results in mice that are already obese (19). In humans, there is a growing number of studies in different fields involving TRE recently.

Since high BMI is a high-risk factor for multiple health problems, the purpose of this article is to assess the effect of TRE studies on changes in body weight and fat mass (primary outcome) and changes in other anthropometric and metabolic variables (secondary outcomes) in adults with overweight or obesity. We used standard cutoff points of BMI to define overweight (BMI, 25–29) and obesity (BMI, ≥30).

Abbreviations: TRE, time-restricted eating; RCTs, randomized controlled trials; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance; WC, waist circumference, MD, mean difference; CI, confidential interval.

# 2. Materials and methods

## 2.1. Protocol and registration

The systematic review was conducted as per PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (20). **Figure 1** shows the pattern of study selection.

The protocol has been registered at Prospero: CRD42022361240.

## 2.2. Inclusion and exclusion criteria

Only randomized controlled trials (RCTs) that met the following criteria were included: the study participants were, 18 years old and above with a body mass index (BMI) greater than 25 kg/m$^2$. The intervention of the experimental group was TRE in which all participants were restricted to eating within an eating window from 8 to 12 h while the control group was not restricted by diet time. The participants were allowed to eat *ad libitum* or follow a hypocaloric diet as long as they followed the same diet in the same trial. There were no restrictions based on sex, race, or country.

The exclusion criteria involved studies (a) not RCT, (b) combined with other interventions, (c) participants with diseases impacting on outcomes, (d) without quantitative outcomes, and (e) duplicate publications.

## 2.3. Search strategy

We searched PubMed (National Library of Medicine), Embase, and Cochrane Central Register of Controlled Clinical Trials for studies published from inception to 23 August 2022, following the PICO (participants, interventions, comparisons, outcomes) principles. We also searched the gray literature on ClinicalTrials.gov, OpenGrey.eu, and Greylit to reduce publication bias. There was no language restriction.

The Medical Subject Headings (MeSH) along with keyword terms utilized were "fasting" or "Intermittent Fasting" or "time-restricted feeding" or "time-restricted eating" and "overweight" or "obesity" or "obese" and "adult" and "random" or "trial."

## 2.4. Study selection and data extraction

Two authors (WC and LB) independently screened the titles and abstracts of the publications identified in the search and relevant articles were retrieved as full texts. If there were different opinions on the inclusion or exclusion of studies, a third author (HZ) would contribute to the discussion and arrive at a consensus result. Where there was missing data, we contacted the authors for additional information. If data is not shown in the text but is available in the supplementary material, it will be extracted in the supplementary material but we give priority to the content of the text.

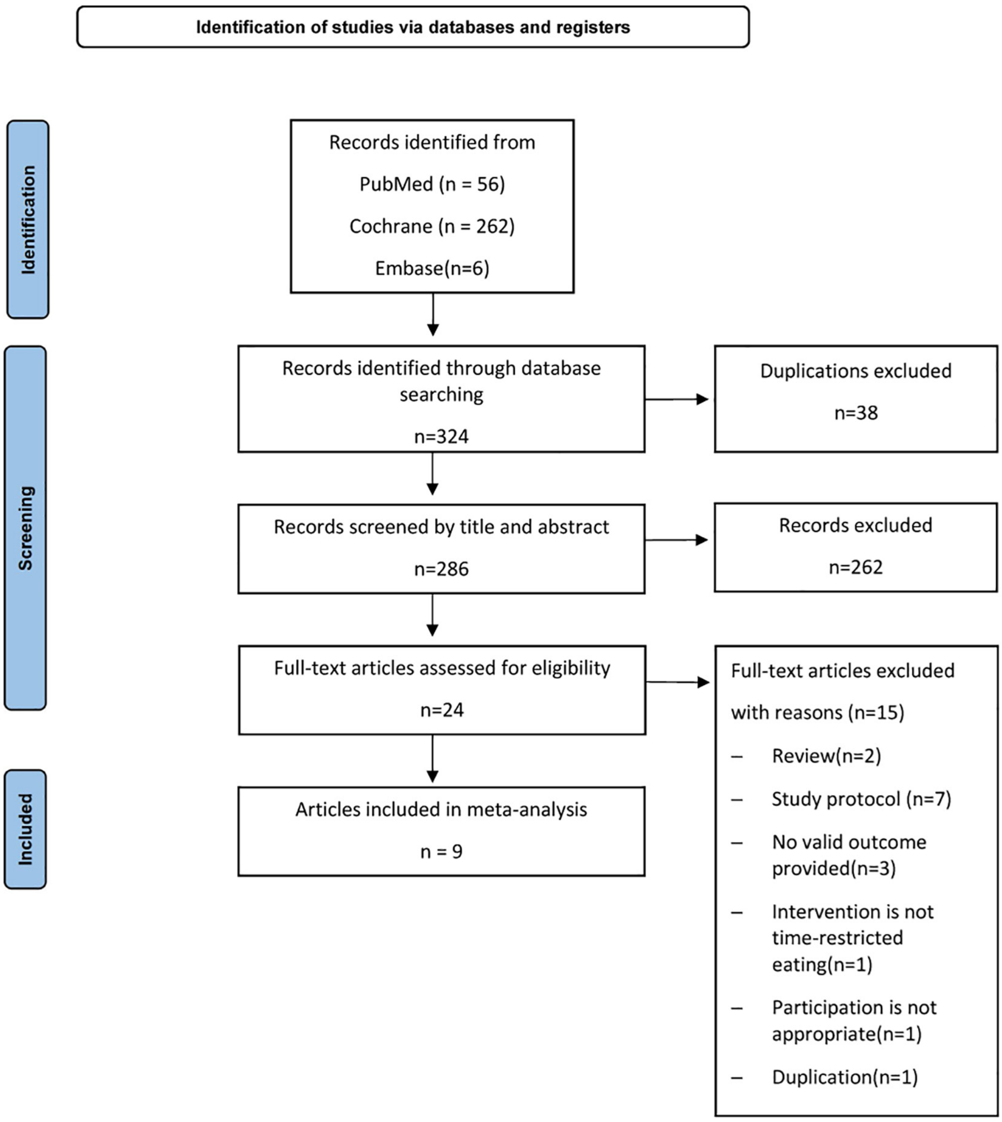


FIGURE 1
PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram.

Where multiple analyses (intention to treat or per-protocol) were reported by the authors, more conservative analyses of intention to treat were extracted, but where the abandonment rate exceeded 45%, protocol analyses were used (21). Besides, when different analysis methods were used for the text and the supplementary material, we gave preference to the analysis method of the text (22).

Two authors (WC and XL) extracted data independently *via* Microsoft Excel 2021. One author (HZ) supervised the selection along with the data abstraction process. The following information was collected from each included study (1) first author name and year of publication; (2) age; (3) baseline BMI; (4) the number of individuals enrolled in each group; (5) duration of eating window; (6) study duration; (7) diet restriction; (8) outcome measurement; (9) study attrition; and (10) the following human variation parameters: weight, BMI, fat mass, lean mass, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), fasting glucose, fasting insulin, homeostasis model assessment-insulin resistance (HOMA-IR), and waist circumference (WC).

## 2.5. Study risk of bias assessment

We used the "Revised Cochrane risk-of-bias tool for randomized trials (ROB-2.0)" to assess the quality of RCTs (23). Bias was assessed as a judgment (high, low, or unclear) for elements from five domains: (1) randomization process; (2) deviations from intended interventions; (3) missing outcome data; (4) measurement of the outcome; and (5) selection of the reported result. All the authors independently participated in the quality assessment and agreed with the results.

## 2.6. Statistical analysis

Both quantitative synthesis and subgroup analysis were performed with Review Manager version 5.4.1 (24). The pooled effect sizes were expressed as mean difference (MD) with a 95% confidence interval (95% CI). $p < 0.05$ indicates a statistical significance. If the heterogeneity was relatively low ($I^2 < 50\%$), we used a fixed

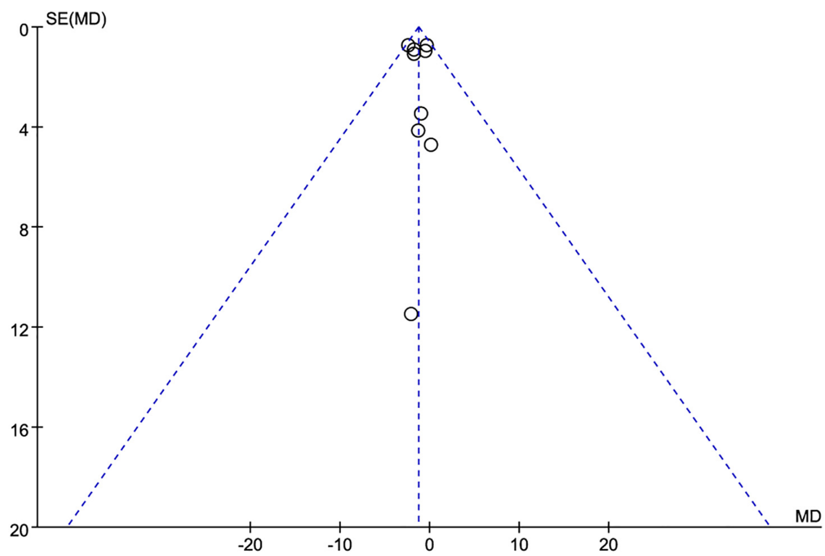


FIGURE 2
Funnel plot for publication bias detection on weight loss changes. MD, mean difference; SE, standard error.

effects model, otherwise a random effects model was applied. For the analysis of all parameters, we use the change between the end of intervention and baseline. If the standard deviation of the change from the baseline is not given in the original research, we assumed an intraparticipant correlation of 0.5 from baseline to follow-up measurements to calculate missing values according to the recommendations in the Cochrane Handbook.

Meanwhile, the subgroup analysis was conducted according to the duration of the eating window (8 vs. >8 h), study duration (<12 vs. ≥12 weeks), and energy intake (with restriction or eat *ad libitum*) in participants. Publication bias was examined with a funnel plot asymmetry (**Figure 2**) and Egger's test. The results of at least four studies were analyzed for data synthesis.

# 3. Results

## 3.1. Study selection

The literature search yielded 324 articles and excluded 38 duplicate studies from the beginning until 23 August 2022. A total of 286 studies were available for a review of titles and abstracts, of which 24 were fully reviewed. A total of 15 studies were excluded 2 reviews, 7 protocols, 3 studies without valid outcomes, 2 studies with inappropriate intervention or participants, and 1 duplication as shown in **Figure 1**. Nine studies (21, 22, 25–31) (665 participants) met the inclusion criteria and were identified and underwent systematic review and data synthesis.

## 3.2.Study characteristics

All included studies were conducted on adults aged 18 years and above whose BMI was more than 25 kg/m$^2$. The largest study enrolled 139 participants (27) whereas the smallest 20 participants (31). Only the duration of the eating window differed between the TRE and control groups in the same study, the rest of the interventions (e.g., exercise and calorie restriction) were the same. Participants in three studies (26, 29, 30) were allowed to eat *ad libitum*. Participants in six studies (21, 22, 25, 27, 28, 31) were advised to follow a calorie-restricted diet, one of which was to be combined with exercise (28). All the studies included an intervention group with a TRE duration of 8 h (22, 26–30), 10 h (25, 31), and 12 h (21). For the control groups, the eating window was ≤12 h (22, 28, 31) or with no restriction (21, 25–27, 29, 30). The duration of the intervention was 8 weeks (22, 29, 31), 12 weeks (26, 30), 14 weeks (28), 39 weeks (25), and 12 months (21, 27). The results of one study (31) were measured by the participants themselves at home, and the rest were objectively measured.

The characteristics of the literature chosen for quantitative synthesis are listed in **Table 1**.

## 3.3. Risk of bias assessment

A graphic summarizing the risk of bias was produced from discussions among the authors, as shown in **Figure 3**. Five studies (22, 26, 27, 29, 30) had low risk of bias. Two studies (25, 28) had some concerns of bias risk due to missing data from moderate dropout rates. One study (21) had high risk of bias due to high dropout rate (53.4%) from long intervention period. One study (31) was categorized as having a high risk of measurement of the outcome because the study procedures and assessments were conducted by participants at home. The study also had some concerns about the risk of bias in random processes, because an equivalent number of men were assigned to each group.

## 3.4. Efficacy

### 3.4.1. Weight and BMI

Nine studies (21, 22, 25–31) (623 individuals, 319 in the TRE group, 304 in the control group) analyzed weight as an

TABLE 1 General features of the nine included articles.

| References | Country | Age | BMI | Sample size | | Duration of eating windows | | Study duration | Diet restriction |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TRE group | Control group | TRE group | Control group | | |
| Isenmann et al. (29) | Germany | 20–40 | 25–33 | 18 (10F) | 17 (11F) | 8 h | No restriction | 8 weeks | The participants were allowed to eat *ad libitum* |
| Chow et al. (30) | USA | 45.5 ± 12.1 | ≥25 | 11 (9F) | 9 (8F) | 8 h | No restriction | 12 weeks | The participants were allowed to eat *ad libitum* |
| Jamshed et al. (28) | USA | 43 ± 11 | 39.6 ± 6.7 | 45 (35F) | 45 (37F) | 8 h | ≥12 h | 14 weeks | The participants were counseled to follow a hypocaloric diet (500 kcal/day below their resting energy expenditure) and exercise 75–150 min/week |
| Liu et al. (27) | China | 31.9 ± 9.0 | 28–45 | 69 (33F) | 70 (35F) | 8 h | No restriction | 12 months | All the participants were instructed to follow a calorie-restricted diet that consisted of 1,500–1,800 kcal/day for men and 1,200–1,500 kcal/day for women |
| Thomas et al. (25) | USA | 38.0 ± 7.8 | 34.1 ± 5.7 | 41 (34F) | 40 (35F) | 10 h | No restriction | 39 weeks | Caloric restriction for both groups |
| de Oliveira Maranhão Pureza et al. (21) | Brazil | 19–44 | 33.3 ± 4.1 | 31F | 27F | 12 h | No restriction | 12 months | All the participants were instructed to follow a hypo-energetic diet |
| Lowe et al. (26) | USA | 46.5 ± 10.5 | 32.7 ± 4.2 | 59 (24F) | 57 (22F) | 8 h | No restriction | 12 weeks | The participants were allowed to eat *ad libitum* |
| Peeke et al. (31) | USA | 18–65 | ≥30 | 39 | 39 | 10 h | 12 h | 8 weeks | Both groups were reduced in energy relative to expenditure for baseline body weight (approximately 500–1,000 kcal/day deficit) |
| Queiroz et al. (22) | Brazil | 30 ± 6 | 30.5 ± 2.7 | 32 | 16 | 8 h | 12 h | 8 weeks | Participants were prescribed a diet plan to promote weight loss, but no food was provided. Energy intake was calculated based on each individual resting metabolic rate multiplied by the physical activity levels of 1.4, −25% of the daily energy requirements |

| References | Outcome measurement | Study attrition |
|---|---|---|
| Isenmann et al. (29) | Weight and anthropometric parameters were measured objectively four times (at the beginning and end of the familiarization phase; the end of the intervention; 6 weeks after the intervention). | 42 participants 7 dropped out at the familiarization phase, 35 completed the study |
| Chow et al. (30) | Body weight, composition, and metabolic outcomes were measured pre and end-intervention objectively. | 22 participants, 20 completed |
| Jamshed et al. (28) | Bodyweight was measured in the non-fasting state in the clinic every 2 weeks throughout the trial. Additional outcomes were measured at week 0 and week 14 objectively. | 90 participants, 59 completed |
| Liu et al. (27) | The outcomes were quantified objectively at baseline and 12 months. | 139 participants, 118 completed |
| Thomas et al. (25) | Objective clinic weights and body composition measurements were obtained at baseline and 39 weeks.[a] | 81 participants, 63 completed |
| de Oliveira Maranhão Pureza et al. (21) | The results were measured before and after, 4, 6, and, 12 months of intervention objectively. | 58 participants, 27 completed |
| Lowe et al. (26) | All participants had their weight measured at home using a Bluetooth scale, which was linked to the research platform. 46 participants completed extensive in-person metabolic testing in Clinical Research Center. | 116 participants, 105 completed |
| Peeke et al. (31) | Study supplies (scale, glucometer, lancets, and glucose strips) were shipped to the participant's homes, and study procedures and assessments were conducted by participants at home. | 78 participants, 60 completed |
| Queiroz et al. (22) | Body weight and anthropometric outcomes were measured in the laboratory by the same experienced researchers. | 48 participants, 37 completed |

Mean ± SD, range; F, female. [a]Home scale weights were obtained in one cohort ($n$ = 26) from week 6 to week 12.

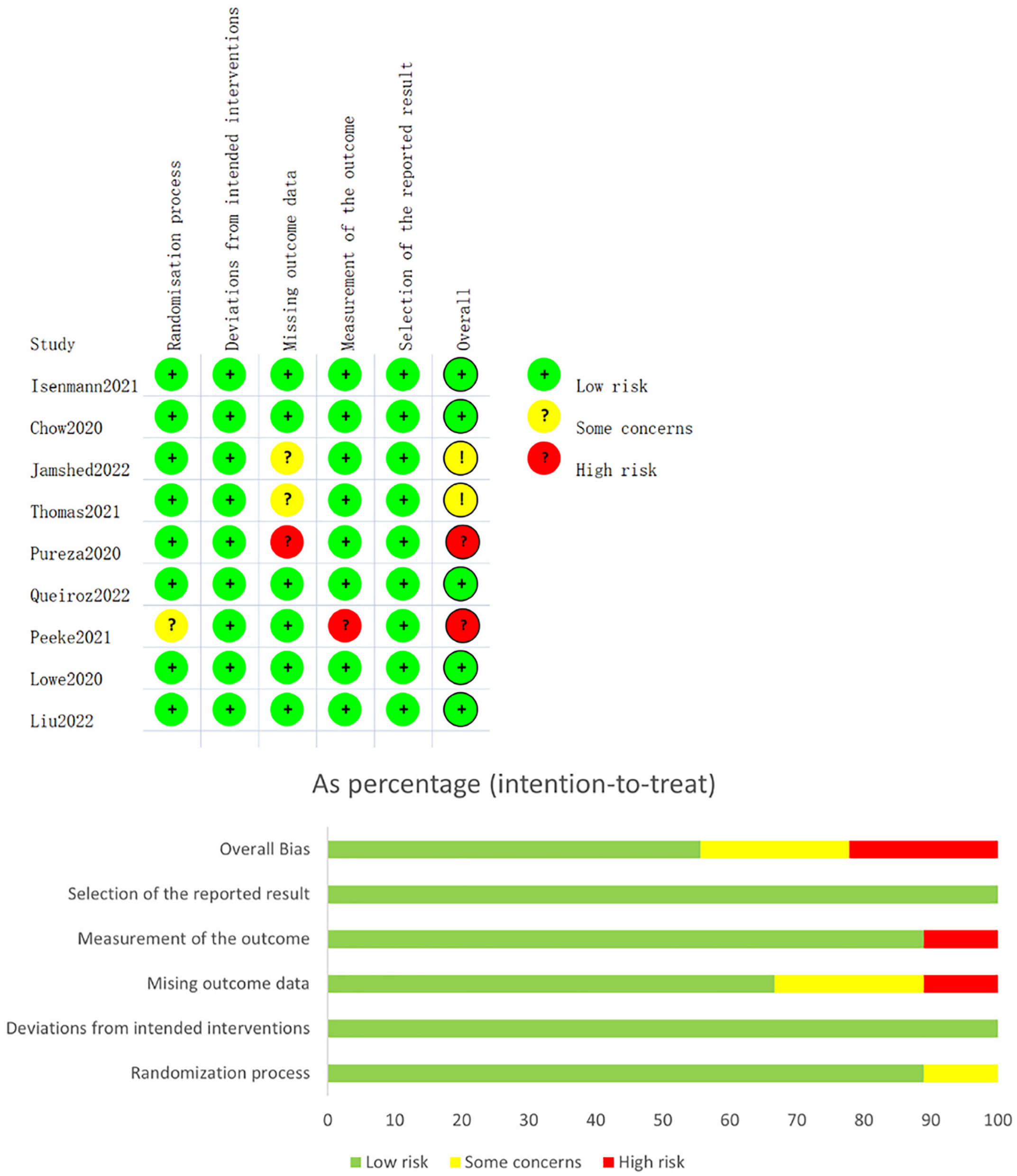


FIGURE 3
Risk of bias assessment.

outcome. Individuals assigned to the TRE intervention group showed a significant weight reduction compared to the control group (−1.28 kg; 95% CI [−2.05, −0.52]; $p$ = 0.001; $I^2$ = 0%) (**Figure 4A**). No significant publication bias was detected by Egger's test ($p$ = 0.543).

Five studies (21, 22, 26, 27, 29) (288 individuals, 149 in the TRE group, 139 in the control group) analyzed BMI as an outcome. Participants allocated to the TRE group showed a significant reduction in BMI compared to the control group (−0.34 kg/m$^2$; 95% CI [−0.64, 0.04]; $p$ = 0.03; $I^2$ = 0%) (**Figure 4B**).

### 3.4.2. Fat mass and lean mass

Seven studies (22, 25–30) included fat mass as an outcome, with 452 individuals (233 in the TRE group, 219 in the control group) evaluated. It demonstrated that the TRE group showed a slight difference in fat mass compared to the control group (−0.72 kg; 95% CI [1.40, −0.03], $p$ = 0.04, $I^2$ = 0%) (**Figure 4C**).

Five studies (25–27, 29, 30) included lean mass as an outcome with 325 individuals (164 in the TRE group, 161 in the control group) evaluated. It demonstrated that there was no difference in lean mass between groups (−0.25 kg; 95% CI [−0.72, 0.22], $p$ = 0.30; $I^2$ = 0%) (**Figure 4D**).

### 3.4.3. Blood pressure

Five studies (21, 26–28, 30) (326 individuals, 163 in the TRE group, and 163 in the control group) analyzed SBP and DBP as the outcome. The TRE group showed a statistically significant reduction in DBP (−2.26 mmHg; 95% CI [−4.02, −0.50], $p$ = 0.01, $I^2$ = 0%) compared to the control group, however, there was no difference

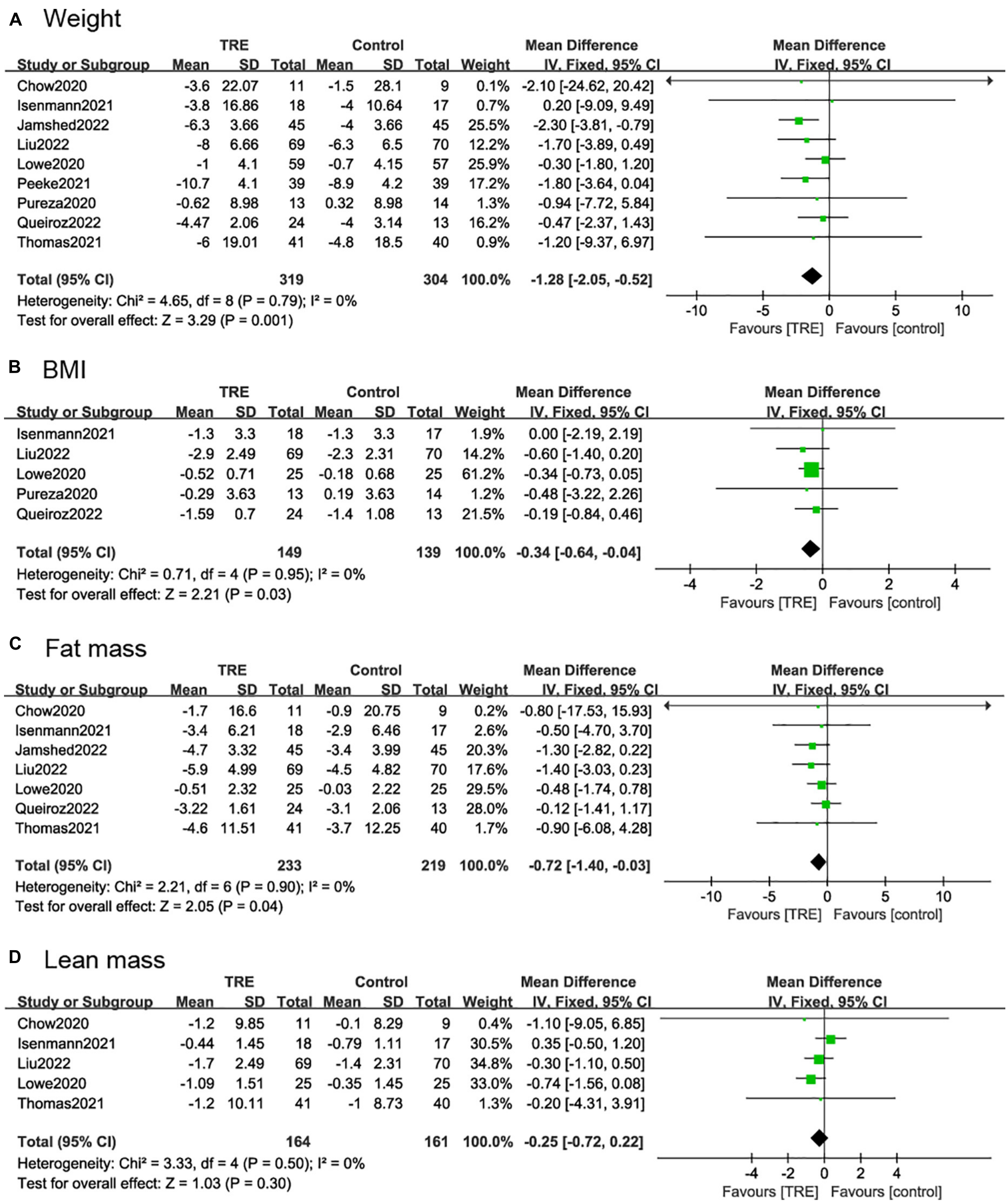


FIGURE 4
Forest plots of comparisons between TRE and the control groups in **(A)** weight; **(B)** body mass index (BMI); **(C)** fat mass; and **(D)** lean mass.

in SBP (−0.75 mmHg; 95% CI [−3.14, 1.63], $p$ = 0.54, $I^2$ = 0%) (**Figures 5A, B**).

### 3.4.4. Fasting glucose, insulin, and HOMA-IR

Six studies (22, 26–28, 30, 31) (364 individuals, 213 in the TRE group, 201 in the control group) tested fasting glucose for results. It demonstrated that there was no difference in fasting glucose levels between groups (−0.49 mg/dl; 95% CI [−2.72, 1.74], $p$ = 0.67, $I^2$ = 0%) (**Figure 5C**).

Five studies (22, 26–28, 30) (197 individuals, 105 in the TRE group, 92 in the control group) analyzed fasting insulin as an outcome. Individuals in the TRE group showed no differences in fasting insulin levels compared to the control group (−0.94 mU/L; 95% CI [−2.98, 1.10], $p$ = 0.37 $I^2$ = 0%) (**Figure 5D**).

Five studies (22, 26–28, 30) (347 individuals, 182 in the TRE group, 165 in the control group) analyzed HOMA-IR as an outcome. It demonstrated that there was no difference in HOMA-IR between groups (−0.33; 95% CI [−0.77, 0.10], $p$ = 0.13, $I^2$ = 0%) (**Figure 5E**).

### 3.4.5. Total cholesterol and triglycerides

Four studies (22, 26–28) (316 individuals, 163 in the TRE group, 153 in the control group) analyzed total cholesterol as an outcome. Individuals in the TRE group did not show differences in total

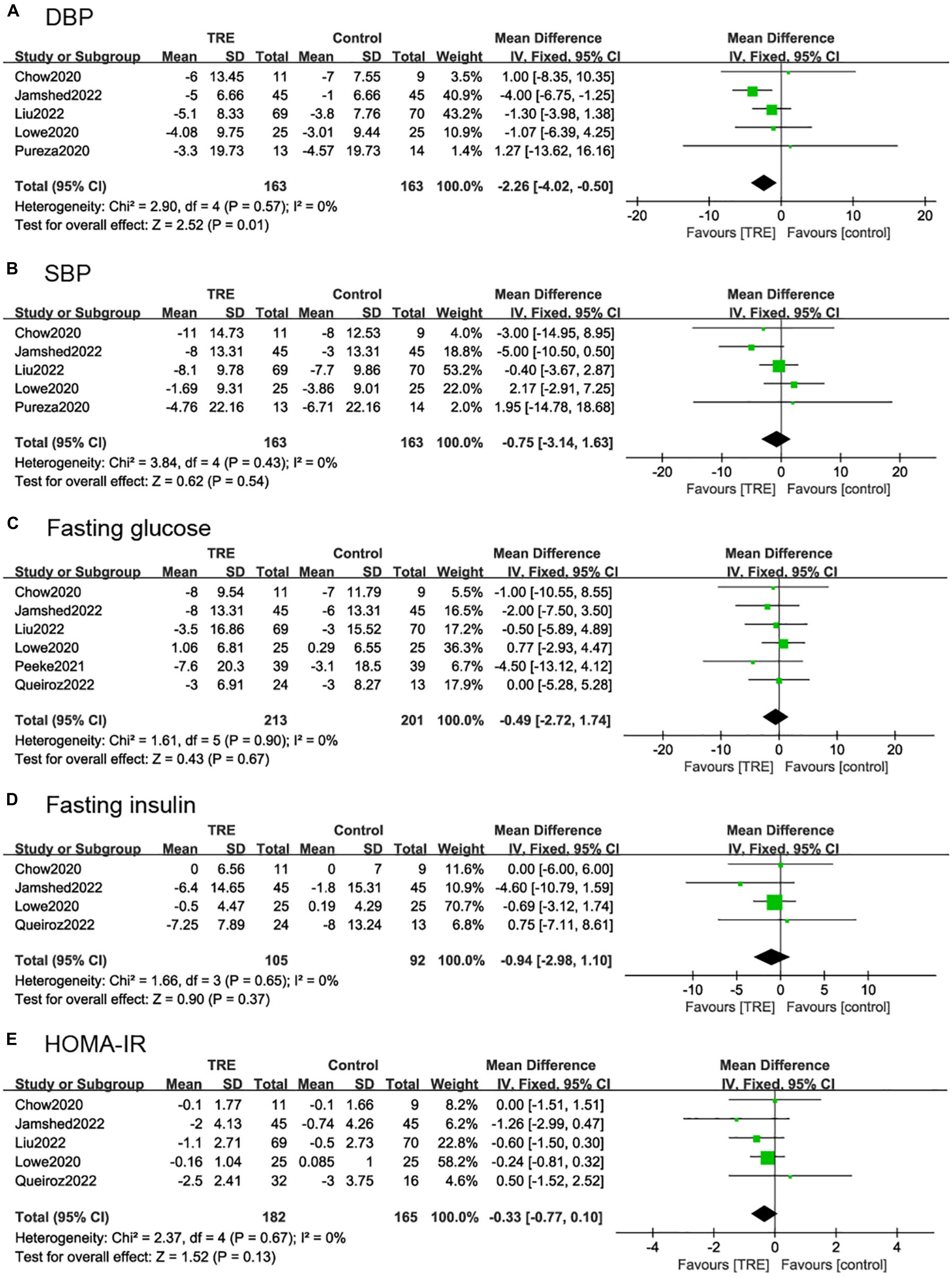


FIGURE 5
Forest plots of comparisons between TRE and the control groups in **(A)** diastolic blood pressure (DBP), **(B)** systolic blood pressure (SBP), **(C)** fasting glucose, **(D)** fasting insulin, and **(E)** homeostasis model assessment-insulin resistance (HOMA-IR).

cholesterol compared to the control group (2.12 mg/dl; 95% CI [−4.46, 8.71], $p = 0.53$, $I^2 = 0\%$) (**Figure 6A**).

Five studies (22, 26–28, 30) (347 individuals, 182 in the TRE group, 165 in the control group) analyzed triglycerides as an outcome. Individuals in the TRE group showed no differences in triglyceride levels compared to the control group (−3.97 mg/dl; 95% CI [−14.48, 6.55] $p = 0.46$, $I^2 = 46\%$). In sensitivity analyses, when removing the study by Chow et al. (30), the heterogeneity decreased to $I^2 = 0\%$, however, the results were still not statistically significant (−0.05 mg/dl; 95% CI [−11.06, 11.05] $p = 0.99$ $I^2 = 0\%$) (**Figure 6B**).

### 3.4.6. HDL and LDL

Five studies (22, 26–28, 30) reported HDL and LDL, with 336 individuals (174 in the TRE group, 162 in the control group) evaluated. The results demonstrated that there was no statistical difference between TRE and control groups in terms of HDL

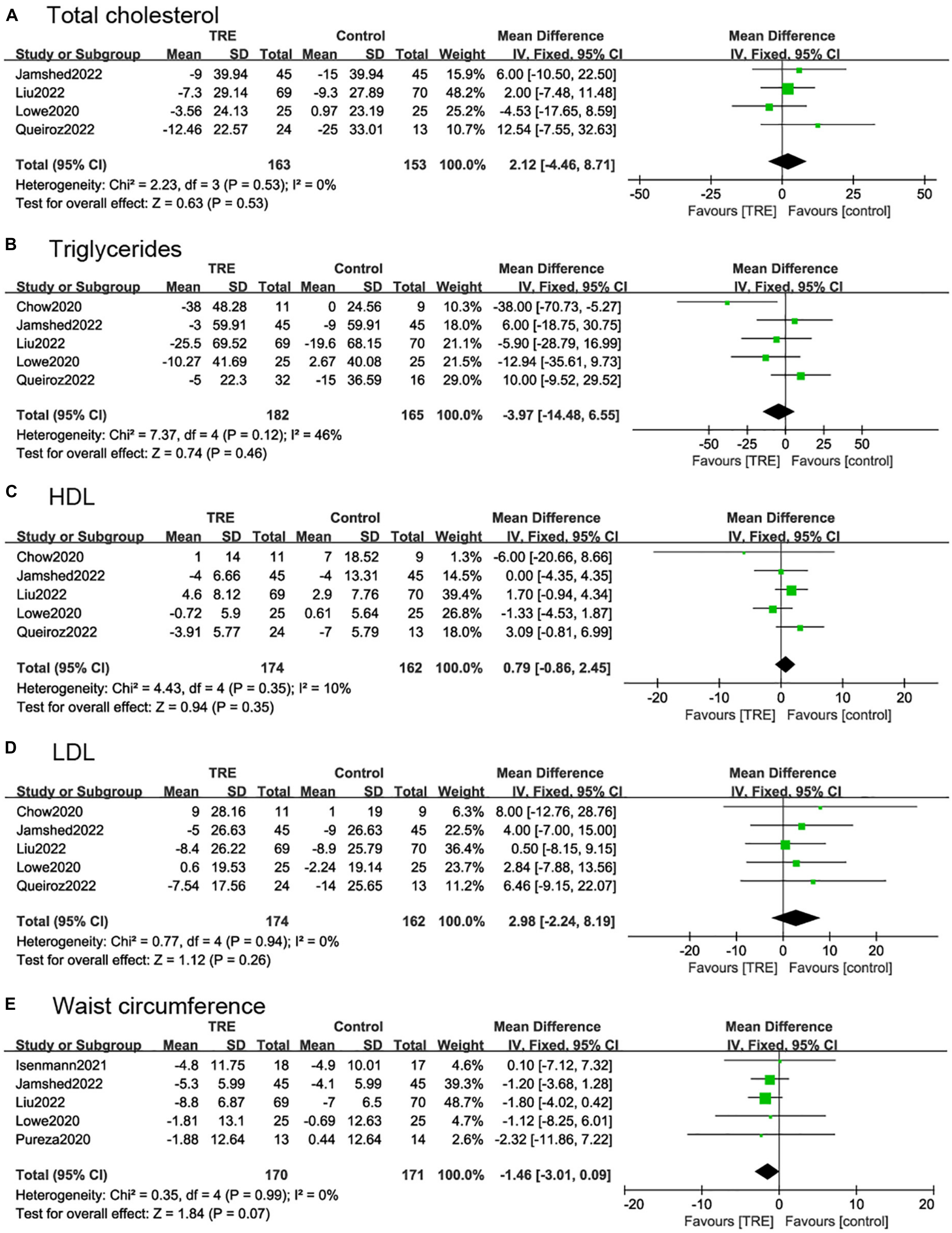


FIGURE 6
Forest plots of comparisons between TRE and the control groups in **(A)** total cholesterol; **(B)** triglycerides; **(C)** high-density lipoprotein (HDL); **(D)** low-density lipoprotein (LDL); and **(E)** waist circumference.

(0.79 mg/dl; 95% CI [−0.86, 2.45], $p = 0.35$, $I^2 = 0\%$) and LDL (2.98 mg/dl; 95% CI [−2.24, 8.19], $p = 0.26$, $I^2 = 0\%$) (Figures 6C, D).

### 3.4.7. Waist circumference

Five studies (21, 26–29) (341 individuals, 170 in the TRE group, 171 in the control group) analyzed WC as an outcome. These demonstrated that TRE had a small effect on WC but with no statistical difference compared with control groups (−1.46 cm; 95% CI [−3.01, 0.09], $p = 0.07$, $I^2 = 0\%$) (Figure 6E).

## 3.5. Subgroup analysis

### 3.5.1. Eating window

According to the duration of the eating window, we divided the studies into two subgroups, 8 h (22, 26–30) and over 8 h (21, 25,

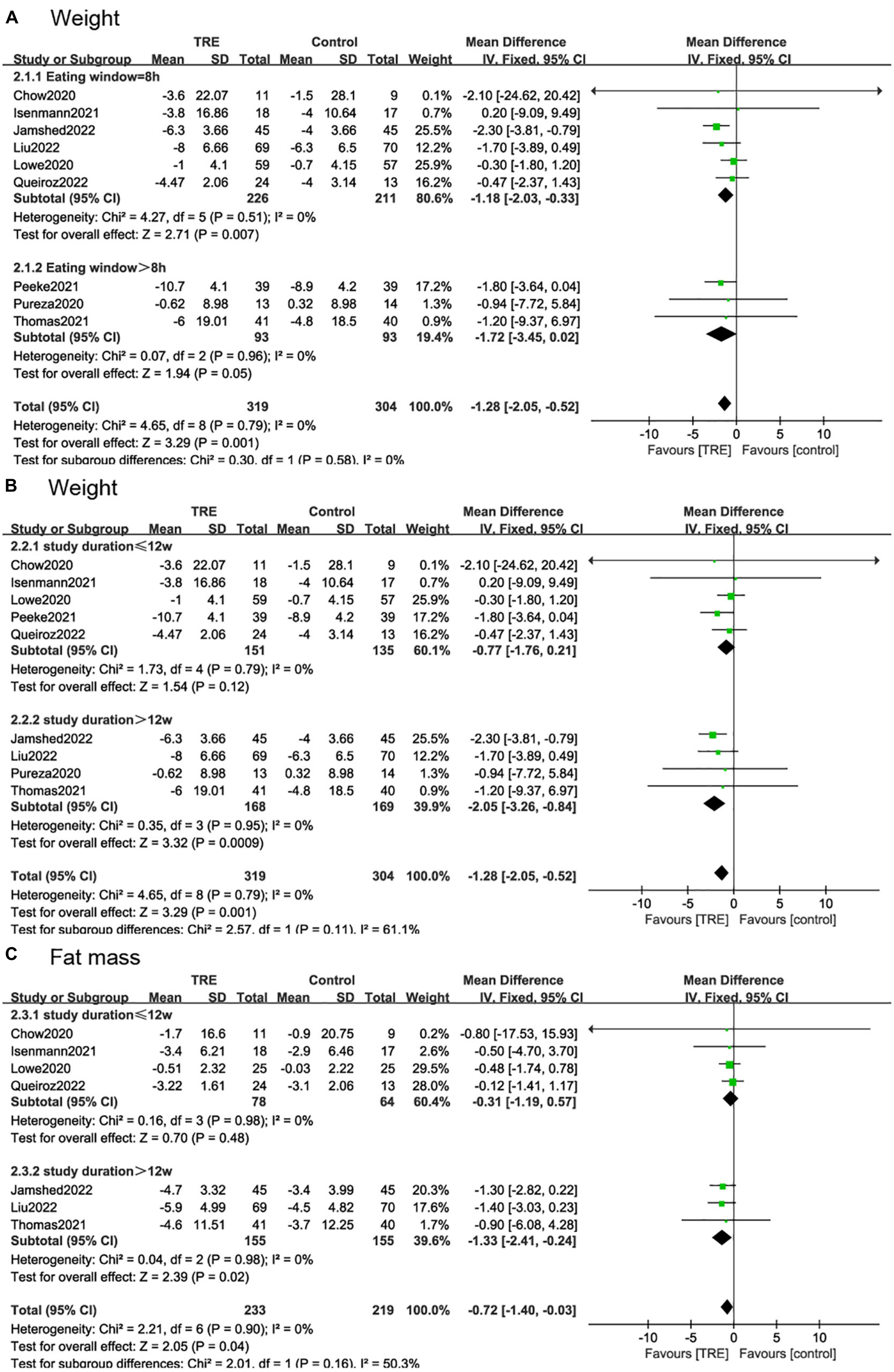


FIGURE 7

Forest plot of **(A)** weight change under the eating window in subgroups of 8 h (2.1.1) and over-8 h (2.1.2); **(B)** weight change under the study durations in subgroups of ≤12 weeks (2.2.1) and >12 weeks (2.2.2); and **(C)** fat mass change under the study durations in subgroups of ≤12 weeks (2.3.1) and >12 weeks (2.3.2).

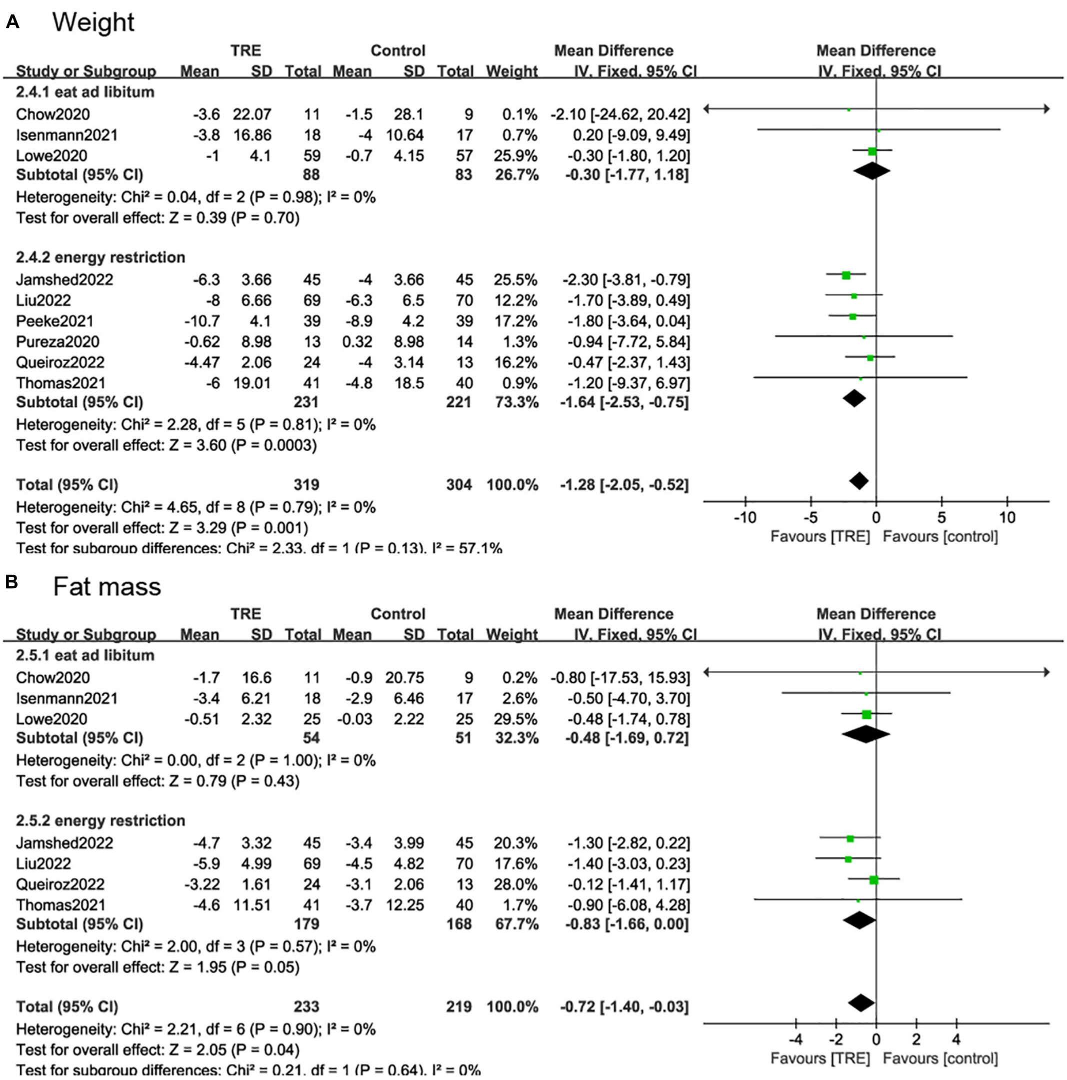


FIGURE 8
Forest plot of **(A)** weight change in subgroups of participants allowed to eat libitum (2.4.1) and participates with caloric restriction (2.4.2); and **(B)** fat mass change in subgroups of participants allowed to eat libitum (2.5.1) and participates with caloric restriction (2.5.2).

31). The 8-h eating window showed a significant weight reduction (−1.18 kg; 95% CI [−2.03, −0.33], $p = 0.007$, $I^2 = 0\%$) compared to the control group while over 8-h eating window showed no statistical difference (−1.72 kg; 95% CI [−3.45, 0.02], $p = 0.05$, $I^2 = 0\%$) (**Figure 7A**).

### 3.5.2. Study duration

In the subgroup analysis for the study duration, we divided the studies into two subgroups. In studies ≤12 weeks (22, 26, 29–31), weight change (−0.77 kg; 95% CI [−1.76, 0.21], $p = 0.12$, $I^2 = 0\%$) and fat mass reduction (−0.31 kg, 95% CI [−1.19, 0.57], $p = 0.48$, $I^2 = 0\%$) showed no difference in the TRE group compared to the control group. However, in studies >12 weeks (21, 25, 27, 28), these two indicators showed a statistically significant difference, with weight (−2.05 kg; 95% CI [−3.26, −0.84], $p = 0.0009$, $I^2 = 0\%$) and fat mass (−1.33 kg; 95% CI [−2.41, −0.24], $p = 0.02$, $I^2 = 0\%$) (**Figures 7B, C**).

### 3.5.3. Energy restriction

We divided the study into two subgroups based on whether there were energy restrictions on the participants. In studies where participants were allowed to eat libitum (26, 29, 30), and there was no difference in weight (−0.3 kg; 95% CI [−1.77, 1.18], $p = 0.70$, $I^2 = 0\%$) and fat mass (−0.48 kg; 95% CI [−1.69, 0.72], $p = 0.43$, $I^2 = 0\%$) between the TRE and the control group. In the studies with caloric restriction (21, 22, 25, 27, 28, 31), there was a significant weight reduction (−1.64 kg; 95% CI [−2.53, −0.75], $p = 0.0003$, $I^2 = 0\%$) in the TRE group compared to the control group. In terms of fat mass, the TRE group also showed a beneficial effect (−0.83 kg; 95% CI [−1.66, −0.00], $p = 0.05$, $I^2 = 0\%$) (**Figures 8A, B**).

## 3.6. Adverse events

One study (27) reported mild adverse events such as fatigue, dizziness, headache, decreased appetite, upper abdominal pain, dyspepsia, and constipation, but the occurrences were similar in TRE and control groups. Hunger and headaches were observed in the TRE group in one study in the first few weeks, but tended to disappear over time (22).

Time-restricted eating regimen was reported to affect daily physical activities and psychological conditions. In one study, a significant reduction in daily movement and step count were observed in the TRE group (26). But in another study, there were no between-group differences in self-reported physical activity and the TRE intervention was more effective at improving total mood disturbances (28).

## 4. Discussion

The present review and meta-analysis included nine studies, with 665 individuals in total. We found a significant decrease in body weight, fat mass, BMI, and DBP compared to the control group. However, there were no changes in lean mass, SBP, WC, insulin, glucose, HOMA-IR, and lipid profile (triacylglycerol, HDL, LDL, and total cholesterol). Although an article on the impact of TRE on people with obesity has been published before (32), several high-quality RCTs addressing the effects of TRE on obese populations emerged later (22, 25, 27, 28), and some are on the way, therefore, this study was necessary.

Through subgroup analysis, we found that the duration of the study had a significant effect on weight change as well as fat mass. In studies longer than 12 weeks, weight change (−2.05 kg; 95% CI [−3.26, −0.84], $p = 0.0009$, $I^2 = 0\%$) and fat mass reduction (−1.33 kg, 95% CI [−2.41, −0.24], $p = 0.02$, $I^2 = 0\%$) were more significant in the TRE group compared to the control group. And in studies less than 12 weeks, those two outcomes did show no difference in the TRE and control groups. However, in a previous meta-analysis (32), the results were that short-term TRE intervention was more effective, which is contrary to the findings of this study. This may be due to the emergence of more RCTs with more participants.

In a subgroup analysis on the duration of the eating window, we also found out that the duration of the eating window was essential to the effect of weight. The 8-h eating window was more effective in weight reduction (−1.18 kg; 95% CI, [−2.03, −0.33], $p = 0.007$, $I^2 = 0\%$) compared with the control group. The over-8 h eating window was also more TRE favorable, but it was not statistically different compared to the control group (−1.72 kg; 95% CI [−3.45, 0.02], $p = 0.05$, $I^2 = 0\%$). This indicates that it would be more useful if limit the length of eating time in the TRE method. Excessive daily eating time will reduce the effect of weight loss. However, compressing the diet to less than 8 h will make adherence more difficult and reduce compliance over the long-term, due to occasional social needs in the evening, which may prolong the eating time. Achieving a balance between improved adherence and good results is the key to following TRE.

In the subgroup analysis targeting energy intake, we found that the studies with caloric restriction (21, 22, 25, 27, 28, 31) had a significant benefit in both weight and fat loss in the TRE group compared to the control group. But in trials (26, 29, 30) where participants were allowed to eat *ad libitum*, there was no statistical difference in weight change. However, due to the low proportion of participants who eat *ad libitum* (26.6% on weight analysis and 23.2% on fat mass analysis), caution is needed when recommending TRE combined with energy restriction.

Time-restricted eating has been approved to be effective in preventing obesity and improving metabolic outcomes in several animal models of obesity. Mice under TRE (food access restricted to 8–10 h) consume equivalent calories as those with *ad libitum* access yet were protected from excessive weight, hyperinsulinemia, inflammation, and hepatic steatosis and have improved motor coordination (18, 33). Rats under TRE also had lower weight gain and adiposity than those on the matched *ad libitum* diet (34). Remarkably, a study by Chaix et al. (35) showed that most of the benefits of TRE are sex-dependent, the TRE prevents weight gain only in male mice. For humans, it appears that men are more likely to lose weight than women in weight loss attempts (36). In the nine trials we included, there was a preponderance of female participants but no gender-specific analysis was given. Further studies are needed to determine the effects of TRE among men and women separately.

Some human observational studies have shown that more than 50% of adults eat for 15 h or more each day, and they often consume most of their calories later in the day (37, 38). A prospective longitudinal study of 420 overweight patients indicated that people who ate late for lunch lost significantly more weight than those who ate early, pointing to the importance of the time of day when food is consumed (39). Based on these considerations, TRE, a relatively new approach to weight management that has emerged and been the focus of attention for both lay people and scientists in recent years, has proven to be a beneficial strategy for inducing weight loss because it can maintain a consistent daily cycle of eating and fasting that supports circadian rhythms (37). TRE did not need to deliberately instruct participants to limit their total energy intake because there was no intentional energy restriction during TRE, but only a change in the timing of eating, which greatly reduced the difficulty of adherence.

Time-restricted eating is a more reasonable and feasible approach than calorie restriction because there is no deliberate energy restriction, only a change in the timing of the diet. In our pool of studies, the adherence rate averages above 70%, but varies relatively widely, which is the main source of bias, from 46.5% (40) to 90.9% (30). The high dropout rate may be due to the long duration of the experiment and financial reasons (40). And the reason for high adherence is that only participants who demonstrated high adherence during the pre-intervention period were included (30).

We observed that weight loss was accompanied by a decrease in lean body mass in both the TRE and control groups. Loss of lean mass can lead to rapid weight regain as well as many health problems, including the impact on health, the ability to perform activities of daily living, and the potential impact on the emotional and psychological state (38, 41). Losing weight while maintaining lean body mass would be ideal. Exercise has been shown to help offset some of the changes in lean mass experienced with weight loss (42, 43). In addition, augmented protein intake, and dietary supplements such as chromium picolinate are proven to help preserve lean mass during weight loss (44).

Greater adherence to some proven healthy diets such as the Mediterranean diet has been associated with significant improvements in health status (45, 46). The RCTs we included are those with unlimited diets and calorie restrictions but there are currently no RCTs combined with specific healthy eating practices. If TRE is combined with a specific healthy diet, it will be interesting to see how it affects weight loss and reduces the risk of metabolic and cardiovascular disease.

In individuals without overweight or obesity, isoenergetic TRE was related to higher fasting glucose and a bigger impairment of glucose tolerance (47). In people with obesity (48) or metabolic syndrome (6), TRE with *ad libitum* intake did not change fasting glucose, fasting triglycerides, or HOMA-IR. In the healthy individuals, TRE did not show weight loss, but still showed beneficial effects like fat mass reduction (49) which indicates that TRE could be beneficial to the population with or without metabolic dysfunction.

More than half of the trials included in this systematic review were conducted in the US (25, 26, 28, 30, 31). These populations may have different dietary patterns compared with those from other countries, which may contribute to a potential risk of bias. Retaining participants for long-term lifestyle interventions can be difficult and bias is a concern when high dropout rates occur. In addition, all trials included did not analyze males and females separately, and we cannot know whether TRE differs between genders. We recommend more high-quality RCTs conducted in different countries as well as genders. Furthermore, only two studies (22, 27) reported mild adverse events. It is unclear whether adverse events occurred in other studies. We strongly suggest that future human studies take possible adverse events into account.

This meta-analysis included nine RCTs and concentrated on the efficacy of TRE in adults with obesity. In comparison with previous studies, we included some new RCTs and add some outcome indicators. Many limitations of this study should be recognized. Firstly, the number of RCTs is insufficient, the sample size is insufficient, and some studies have a high risk of bias. Secondly, the majority of participants were women, making the results difficult to generalize. Thirdly, most of the studies have a short duration, we cannot know long-term benefits and safety. Finally, the intention-to-treat analyses of RCTs may lead to relatively conservative results. There are still some unfinished experiments on ClinicalTrials.gov (50–52) and when these are completed and included in the meta-analysis, a more definitive conclusion will be drawn.

## 5. Conclusion

We concluded that the TRE regimen seems to have a beneficial effect on weight and fat mass reduction, and improves BMI and DBP, but no significant effects on other metabolic parameters were observed. Subgroup analysis showed that the eating window should not be too long, with better results below 8 h. In addition, TRE combined with calorie restriction may have a better effect, but caution is needed due to the insufficient sample. It is unclear if TRE has the same effect on males and females. We strongly recommend that future human studies take gender issues into account and analyze them separately. Further high-quality RCTs and longer follow-up studies are needed to make clearer conclusions.

## Author contributions

WC and LB: conceptualization and formal analysis. HZ: methodology, validation, supervision, and funding acquisition. XL: software and investigation. LB: resources. WC and XL: data curation and writing—original draft preparation. WC and PY: writing—review and editing. PY: visualization. WC: project administration. All authors had 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.

## Publisher's note



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# The association of energy and macronutrient intake at breakfast and cardiovascular disease in Chinese adults: From a 14-year follow-up cohort study

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**Background:** We aimed to examine the associations between energy and macronutrient intakes at breakfast and the incidence of cardiovascular events among Chinese adults.

**Methods:** There were 12,937 participants from the China Health and Nutrition Survey who met the study criteria and completed six rounds of questionnaires in 1997, 2000, 2004, 2006, 2009, and 2011. Combined weighing methods with 24-h dietary recall were used to measure dietary intake throughout the day. Intakes of macronutrients at breakfast were calculated using energy provided by nutrients as a percentage of breakfast energy. We calculated hazard ratios using a multivariable Cox frailty model with random intercepts to account for household clustering.

**Results:** During follow-up, we documented 453 (3.6 per 1,000 person-years) major cardiovascular events, 195 (1.5 per 1,000 person-years) myocardial infarctions, and 293 (2.3 per 1,000 person-years) strokes. In Chinese adults, more breakfast carbohydrates or less proteins intake was associated with the reduced risk of cardiovascular diseases. Especially for women, higher intake of breakfast carbohydrates was associated with a lower risk of major cardiovascular events (quintile 5 vs. quintile 1, HR 0.47 [95%CI 0.30–0.74]; $p_{trend}$ =0.0008) and stroke (quintile 5 vs. quintile 1, HR 0.48 [95%CI 0.26–0.88]; $p_{trend}$ =0.0006). Higher intake of breakfast proteins was associated with a higher risk of major cardiovascular events (quintile 5 vs. quintile 1, HR 1.77 [95%CI 1.12–2.79]; $p_{trend}$ =0.1162), myocardial infarction (quintile 5 vs. quintile 1, HR 2.49 [95%CI 1.21–5.11]; $p_{trend}$ =0.2641). There was a significant association between breakfast fat intake and cardiovascular diseases in the adult population, but less significant correlation was found in Chinese men or women. Breakfast fat intake was positively associated with the risk of major cardiovascular events (quintile 5 vs. quintile 1, HR 1.74 [95%CI 1.27–2.36]; $p_{trend}$=0.0070), myocardial infarction (quintile 5 vs. quintile 1, HR 2.03 [95%CI 1.23–3.37]; $p_{trend}$=0.0168), and stroke (quintile 5 vs. quintile 1, HR 1.64 [95%CI 1.12–2.41]; $p_{trend}$=0.0732). There was a significant reduction in major cardiovascular events and stroke when breakfast energy intake was moderated, even if the independence of skipping breakfast.

**Conclusion:** High carbohydrate intake and low protein and fat intake at breakfast may contribute to cardiovascular health while maintaining a moderate energy intake.

KEYWORDS

carbohydrate, protein, fat, energy, breakfast, cardiovascular disease, CHNS

# 1. Introduction

In 2018, cardiovascular disease (CVD) mortality in China remained at the top of the disease spectrum (1), accounting for an average of two out of every five deaths. Stroke, as one of the major CVD, has become the leading cause of death among adults in China (2). According to the "China Cardiovascular Health and Disease Report 2020" (1), approximately 12 million people in China are estimated to have strokes, and 11.39 million have coronary heart disease such as myocardial infarction (MI). CVD has rapidly transformed from a disease in developed countries to a global disease with increasing prevalence and incidence in low-income countries. The prevailing risk factors for cardiovascular disease, namely hypertension, and diabetes mellitus, along with the unhealthy poor-quality diet, would contribute to this complex transition (3).

The presence of poor eating habits or dietary factors has been validated to be associated with a range of chronic diseases (4). As advocated by "2021 Dietary Guidance to Improve Cardiovascular Health," a low-quality diet is strongly associated with cardiovascular morbidity and mortality (5). Equally, meal timing as well as daily nutrient intake regulates cardiovascular risk. There was evidence that the timing of food consumption may alter the circadian rhythm of metabolism, which in turn affects the biological clock (6, 7). According to studies, late-lunch eating (8) and late-night eating (9, 10) were related to a higher risk of cardiometabolic health. Similarly, breakfast skipping or irregular breakfast eating habits were associated with a greater risk of CVD (11–13), though a recent review on the association of breakfast skipping with cardiovascular disease has drawn a controversial conclusion (14). However, with the accelerated pace of life and the deep-rooted concept of weight control and weight loss, breakfast skipping, as a part of the strategies proposed for reducing energy intake, is becoming more prevalent (15, 16). The "Survey Report on the Status of Chinese Residents' Breakfast Diet" issued by the Chinese Nutrition Society has shown that more than 30% of Chinese people cannot eat breakfast every day. Noticeably, it cannot be assumed that people who eat breakfast are necessarily healthier. The basic principle of a healthy breakfast is to try to ensure that the variety of food is as diverse as possible and nutrient intake at breakfast is reasonable.

Studies have investigated the beneficial effects of regular eating breakfast on risk factors of cardiovascular disease (11, 17), but subsequent results remain inconsistent, possibly because of different breakfast compositions. A series of studies have supported that total energy consumption at breakfast reduced weight gain and CVD risk factors, such as elevated serum low-density lipoprotein cholesterol (18, 19). An animal study also has made a similar conclusion that high energy intake at breakfast has a favorable regulation of blood lipids (20). There was evidence, both from a randomized controlled trial (21) and a prospective observational study (22), showing that a reduction in dietary saturated fatty acids (SFA) reduces the risk of CVD. While few studies have been conducted that assess whether fat intake at breakfast has any effect on CVD, researchers found a reduction in the risk of intracerebral hemorrhage associated with higher saturated or monounsaturated fat consumption at breakfast in Japanese men (23). Correspondingly, greater protein intake at breakfast could reduce body weight (18, 24), and was inversely associated with systolic and diastolic pressure and positively associated with high-density lipoprotein cholesterol (25), which may have beneficial effects on cardiovascular health. Protein intake from plant sources was associated with a lower risk of cardiovascular disease mortality (26), whereas protein intake from animal sources with a higher risk of cardiovascular health (27). Given to different animal sources, many studies have provided much evidence that red meats, such as poultry and beef, were associated with a range of adverse cardiovascular health (28–30). Furthermore, processed meat that contain high amounts of sodium have been linked with a higher risk of CVD incidence (30).

In most nutritional guidelines, there is a lack of recommendations regarding eating habits (timing, quantity, energy content, and frequency) in adults (31, 32). During the past two decades, the overall energy intake in Chinese adults has shown a decreasing trend, and the dietary structure significantly changed (33). However, a longitudinal study has not been conducted to date to examine the relationship between macronutrient intake at breakfast and the risk of cardiovascular disease. In this study, our primary aim was to assess the association of energy and macronutrient (carbohydrate, protein, and fat) intake at breakfast with cardiovascular disease events among Chinses adults. The secondary aim was to investigate the effect of energy and macronutrient intake at breakfast on cardiovascular diseases in participants with the presence of hypertension.

Abbreviations: CVD, cardiovascular disease; MI, myocardial infarction; SFA, saturated fatty acid; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; MET, metabolic equivalent of task; CHNS, China health and nutrition survey; SD, stand deviations; CI, confidence interval; HR, hazard ratios.

# 2. Materials and methods

## 2.1. Study participants

In this study, data were derived from a stratified, multistage prospective survey of the China Health and Nutrition Survey (CHNS), a cohort study. More details of the sampling process and design used in CHNS have previously been published elsewhere (34). The food codes used before 1997 in CHNS did not match the published China Food Composition tables (35), and the 2015 dietary survey data have not yet been published. In brief, data on 22,418 participants were collected in nine provinces from 1997, 2000, 2004, 2006, 2009, and 2011, including Liaoning, Heilongjiang, Jiangsu, Shandong, Henan, Hubei, Hunan, Guangxi and Guizhou. The following participants were excluded from the present analysis: 6528 participants participated in only one wave; 2,381 individuals aged less than 18 years old at baseline; 238 individuals had a myocardial infarction, or stroke at baseline; 167 individuals had implausible energy intake (<500 or > 5,000 kcal per day); 40 individuals had missing data on smoking, alcohol intake; 127 individuals had a pregnancy. A total of 12,937 individuals were involved in this analysis (Figure 1).

## 2.2. Dietary assessment

Dietary intake was evaluated at the household and individual level using the household food stock method combined with three consecutive 24-h recalls (36, 37), which means selected for 3 consecutive days from Monday to Sunday according to the

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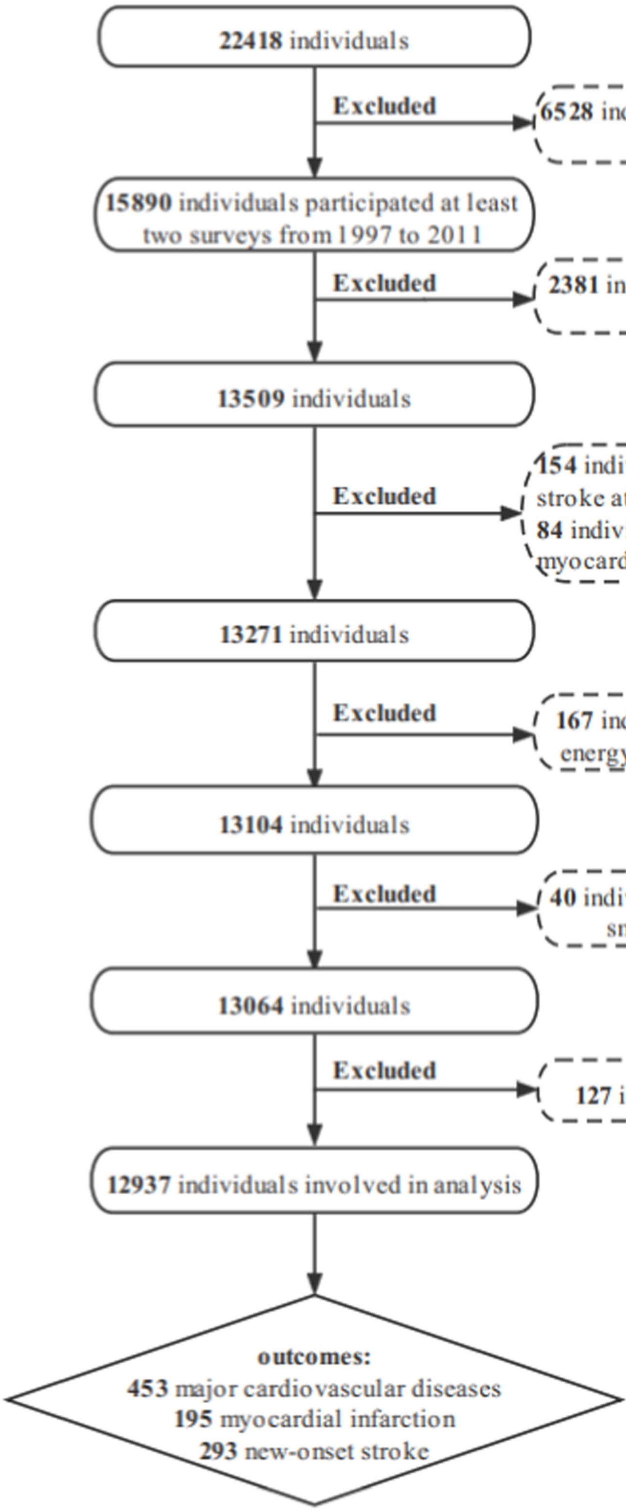


FIGURE 1
Flow chart of sample selection.

randomization principle. Investigators were required to receive standardized training on dietary survey techniques and proper food estimation methods before conducting the household dietary survey. The classification of food categories is based on the food codes recorded in the CHNS database. The food codes in the CHNS database have used two different systems of food coding. In particular, food codes from the Chinese Food Composition Table (1991) were used in 1997 and 2000 to calculate individual daily intake of select nutrients for each food item. From 2004 to 2011, surveys were based on the 2002 edition of the Chinese Food Composition Table with a supplement of the 2004 edition. Noteworthy, different food sources under the same category cannot be directly summed up. The differences in energy and nutrients provided by different food sources should be noted when estimating food consumption, to ensure the accuracy of meal data. A double labeled water method has been validated in this survey to determine total energy intake from combined dietary intake (38). Calculation of the proportion of energy derived from nutrients (carbohydrate, protein, and fat) at breakfast is as follows: for carbohydrate, [carbohydrate intake (g/day) × 4]/breakfast energy intake (kcal/day) × 100; for fat, [fat intake (g/day) × 9]/breakfast energy intake (kcal/day) × 100; for protein, [protein intake (g/day) × 4]/breakfast energy intake (kcal/day) × 100. Additionally, a cumulative method on the averages of energy and macronutrients was applied to provide more weight to individuals' baseline diet (39), and represented long-term habitual dietary intakes.

## 2.3. Health outcomes

Myocardial infarction and stroke onset were obtained by face-to-face or telephone interview according to the study participant's self-report of previous diagnosis and treatment, and only if that diagnosis had been made by a professional physician. When asked, "Has there been a myocardial infarction diagnosed in the past year by a doctor?," a positive answer indicated a myocardial infarction; stroke was defined as a positive answer to the question "Have you been diagnosed with a stroke or transient ischemic attack by a doctor?" Major cardiovascular events included myocardial infarction and stroke. The follow-up period was calculated as the time from their initial year of survey participation to either an expected outcome event or lost to follow-up or the censoring date. As to avoid temporal bias, for example, if an individual participated the first wave in 1997, for cardiovascular outcomes during the 1997–2000 time period, the follow-up period was calculated as the following: [2000–1997 year]/2. Here we summarized the formula as the following:

The follow-up period = $[(Y_{n-1}-Y_1) + (Y_n-Y_{n-1})/2]$, $n \geq 2$.

The six rounds of survey were conducted in 1997, 2000, 2004, 2006, 2009 and 2011. In this formula, n indicates the number of times participated in the survey, $Y_n$ indicates the survey year of n wave, $Y_{n-1}$ indicates the survey year of n-1 wave, $Y_1$ indicates the year of the first participation in the survey.

## 2.4. Confounding variables

Sociodemographic characteristics including age (years), gender (men/women), ethnicity (Han/ethnic minorities), living area (urban/rural), education level (primary school or lower/Lower middle school/Middle school or above), region (Northeast/East Coast/Central/West) and annual household income (Yuan) were assessed in the questionnaires. This study was conducted to determine the presence of hypertension according to hypertension control guidelines (40). Hypertension was determined when one of the following conditions was met: (1) those who self-reported history of diagnosis/treatment of hypertension; (2) those who had taken antihypertensive drugs; (3) systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg at physical measurement. SBP/DBP were checked by trained staff. Diabetes was determined when one of the following conditions was met: (1) those who self-reported having diabetes in the questionnaire; (2) those who had a positive answer to the question "If you had been diagnosed with diabetes, had you used any of the following treatments (i.e., oral hypoglycemic drugs, or Chinese medicine, or insulin injection)?" Body mass index (BMI) was calculated by the formula "BMI = weight (kg)/height ($m^2$)." According to the Chinese BMI reference standard, BMI < 18.5 kg/$m^2$ is defined as underweight, 18.5 ≤ BMI < 24 kg/$m^2$ as healthy weight, 24 ≤ BMI < 28 kg/$m^2$ as overweight, and BMI ≥ 28 kg/$m^2$ as obesity. Physical activity level was assessed based on time spent per week in different occupational, domestic, transportation and leisure activities using a validated questionnaire that calculates the Metabolic Equivalent of Task (MET) for each activity according to the Physical Activity Compendium (41, 42). Smoking status was categorized as a non-smoker, ex-smoker, and current smoker. Alcohol intake was classified on the basis of frequency of alcohol consumption (non-drinker, < 3 times per week and ≥ 3 times per week). Dietary measurements included dietary fiber (g/day), grains (g/day), vegetables (g/day), total energy intake (kcal/day), SFA (g/day); breakfast skipping (yes/no).

## 2.5. Statistical analysis

Continuous variables are summarized as means and stand deviations (SDs) and categorical variables as percentages. We evaluated the proportional hazards assumption using the Schoenfeld residual method combined with visual inspection of log–log plots, which were consistent with proportional hazards. Hazard ratios (HRs) of three cardiovascular outcomes (major cardiovascular events, MI, and stroke) attributed to energy intake including breakfast energy, the proportion of energy from macronutrients at breakfast were calculated using a multivariable Cox frailty model with random intercepts to account for household clustering. For the overall analysis, participants were categorized into quintiles of energy intake at breakfast and nutrient intake (carbohydrate, fat, and protein) at breakfast based on the percentage of energy provided by nutrients. The lowest quintile category was used as the reference group. Minimally adjusted models were adjusted for age, sex, and household clustering as a random effect. Maximally adjusted models were further adjusted for an urban or rural location, education level, income, physical activity, smoking status, alcohol intake, BMI, history of hypertension, diabetes, total energy intake, saturated fatty acid (SFA), grains, vegetables, and dietary fiber. In addition, we used restricted cubic splines with four knots (at the 5th, 35th, 65th, and 95th) to investigate the shape of the association between breakfast nutrient intakes and outcomes. We further built two sets of predicted isocaloric models to estimate the effect of isocaloric replacement (as 5% of energy) of carbohydrates at breakfast with protein and fat intake at breakfast when total energy intake at breakfast was constant (43), simultaneously including energy intake at breakfast and percentages of energy from carbohydrate and other specific macronutrients at breakfast (continuous), as well as other potential confounders. Interactions were explored between energy and macronutrient intake at breakfast and the presence of hypertension. We introduced a cross-product interaction term in the multivariable model to assess the significance of the interaction and to examine whether the effect of energy and macronutrient intake at breakfast on events differed in participants with or without hypertension. The fully adjusted model was subjected to sensitivity analysis by the addition of skipping breakfast. The significance threshold for all comparisons was set at 5% for all *p* values. All statistical analyses were performed with R software, version 4.1. Spline curves were generated with the *"rms"* package.

## 3. Results

### 3.1. Participants' characteristics at baseline and health outcomes

Between 1997 and 2011, a total of 12,937 participants aged 18–93 years old (mean ± SD, 43.2 ± 14.9) at baseline were included and analyzed in this study, of which 6,286 (48.6%) were males. The median follow-up of 11 years (IQR 7–14 years), during which data regarding major cardiovascular events were available for 453 (3.6 per 1,000 person-years) participants, all myocardial infarctions were available for 195 (1.5 per 1,000 person-years) participants and new-onset strokers were available for 293 (2.3 per 1,000 person-years) participants.

Demographic characteristics of participants and data on dietary intake at baseline according to quintiles of energy intake at breakfast are presented in Table 1. Compared with those in quintiles 1–4, participants in the fifth quintile who consumed more energy at breakfast were more likely to be men, poorly educated, those with high physical activity levels, and those who lived in rural areas and were from regions of central China. Participants who were to be no smokers or alcohol drinkers at baseline were more likely to appear in the fifth quintile. Breakfast energy intake was associated with higher intakes of total energy and carbohydrate and lower intakes of protein and fat at baseline.

### 3.2. Association between health outcomes and energy and macronutrients intake

The hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between breakfast energy and macronutrient intake and health outcomes are shown in Table 2. Higher percentage energy from carbohydrate intake at breakfast was associated with a lower risk of major cardiovascular events (quintile 5 vs. quintile 1, HR 0.60 [95%CI 0.44–0.81]; $p_{trend} < 0.0001$), new-onset stroke (quintile 5 vs. quintile 1, HR 0.58 [95%CI 0.40–0.86]; $p_{trend} = 0.0121$). In comparisons between quintile 5 and quintile 1, percentage energy from protein intake at breakfast was associated with a higher risk of major cardiovascular events (quintile 5 vs. quintile 1, HR 1.72 [95%CI 1.25–2.36]; $p_{trend} = 0.0024$), myocardial infarction (quintile 5 vs. quintile 1, HR 1.81 [95%CI 1.09–3.01]; $p_{trend} = 0.0058$), and new-onset stroke (quintile 5 vs. quintile 1, HR 1.53 [95%CI 1.04–2.25]; $p_{trend} = 0.1606$), after multivariable adjustment for covariates. Similarly, percentage energy from the fat intake at breakfast was positively associated with risks of major cardiovascular events (quintile 5 vs. quintile 1, HR 1.74 [95%CI 1.27–2.36]; $p_{trend} = 0.0070$), myocardial infarction (quintile 5 vs. quintile 1, HR 2.03 [95%CI 1.23–3.37]; $p_{trend} = 0.0168$), and new-onset stroke (quintile 5 vs. quintile 1, HR 1.64 [95%CI 1.12–2.41]; $p_{trend} = 0.0732$).

For breakfast energy intake, we observed a possible non-linear relationship between breakfast energy intake and cardiovascular outcomes. In Figure 2, we used a restricted cubic spline to visualize the relationship between the risk of CVD and energy intake at breakfast on a continuous scale. Multivariable-adjusted restricted cubic spline further showed a significant L-shaped association of breakfast energy intake with stroke (Figure 2C). The L-shaped curve indicated that energy intake at breakfast was inversely associated with the risk of stroke at lower intake levels, but higher intakes increased the risk when exceeding a certain threshold. That is to say, breakfast energy intake that is too low or too high might increase the risk of stroke. Correspondingly, the relationship between energy intake at breakfast and major cardiovascular events was mainly U-shaped (Figure 2A), given that the first half of the curve was relatively flat. The U-shaped curve indicated that energy intake at breakfast that is too low or too high might also increase the risk of major cardiovascular events.

In the analysis for energy and major nutrients from breakfast, we evaluated the associations of intakes of energy, carbohydrates, protein, and fat at breakfast with risks of major cardiovascular events, MI, and stroke by sex (Supplementary Table 1). For male participants, adequate intake of energy at breakfast may reduce the risk of stroke (quintile 5 vs. quintile 1, HR 0.56 [95%CI 0.36–0.88]; $p_{trend} = 0.0419$). For female participants, higher percentage energy from carbohydrate intake at breakfast was associated with lower risk of major cardiovascular events (quintile 5 vs. quintile 1, HR 0.47 [95%CI 0.30–0.74]; $p_{trend} = 0.0008$), stroke (quintile 5 vs. quintile 1, HR 0.48 [95%CI 0.26–0.88]; $p_{trend} = 0.0006$). Percentage energy from protein intake at breakfast was associated with higher risk of myocardial infarction (quintile 5 vs. quintile 1, HR 2.49 [95%CI 1.21–5.11]; $p_{trend} = 0.2641$). In addition, breakfast proteins were divided into animal and plant proteins and then compared separately by sex with regard to health outcomes, but no association was found (Supplementary Table 2).

### 3.3. Substitution of carbohydrate intake with protein and fat intake at breakfast and cardiovascular risk

No significant associations with the risk of major cardiovascular disease, MI, and stroke were found for the replacement of carbohydrates at breakfast with protein or fat at breakfast (Figure 3).

### 3.4. Associations with energy and macronutrient intake and health outcomes according to the presence of hypertension

We further examined associations with energy and macronutrient intake and health outcomes according to the presence of hypertension at baseline. There were no significant interactions between breakfast energy intake and cardiovascular events (Figure 4). However, significant interactions were tested between macronutrient intake and a history of hypertension. We conducted stratification analyses to investigate the effect of percentage energy from carbohydrate, protein, and fat intake at breakfast on health outcomes. The higher percentage of energy from carbohydrate intake was associated with a lower risk of major cardiovascular events and new-onset stroke in participants

TABLE 1 Baseline characteristics according to quintiles of energy intake at breakfast: CHNS, 1997–2011.

| Characteristics | Quintile 1 (*n*=2,588)<268.2kcal/d | Quintile 2 (*n*=2,587) 268.2–359.0kcal/d | Quintile 3 (*n*=2,587) 359.0–443.3kcal/d | Quintile 4 (*n*=2,587) 443.3–562.6kcal/d | Quintile 5 (*n*=2,588)>562.6kcal/d | *p* value |
|---|---|---|---|---|---|---|
| Age, years | 43.16 ± 15.10 | 42.96 ± 14.64 | 43.75 ± 14.90 | 43.09 ± 14.83 | 43.29 ± 15.01 | 0.373 |
| Male, % | 1,090 (42.1) | 1,022 (39.5) | 1,179 (45.6) | 1,400 (54.1) | 1,595 (61.6) | < 0.001 |
| Han, % | 2,208 (85.3) | 2,323 (89.8) | 2,327 (89.9) | 2,279 (88.1) | 2,226 (86.0) | < 0.001 |
| Urban residence, % | 875 (33.8) | 869 (33.6) | 960 (37.1) | 927 (35.8) | 881 (34.0) | 0.03 |
| Education | | | | | | |
| Primary school or lower | 1,168 (45.1) | 1,194 (46.2) | 1,152 (44.5) | 1,182 (45.7) | 1,209 (46.7) | < 0.001 |
| Lower middle school | 724 (28.0) | 747 (28.9) | 813 (31.4) | 834 (32.2) | 892 (34.5) | |
| Middle school or above | 696 (26.9) | 646 (25.0) | 622 (24.0) | 571 (22.1) | 487 (18.8) | |
| Region | | | | | | |
| Northeast | 435 (16.8) | 473 (18.3) | 621 (24.0) | 644 (24.9) | 497 (19.2) | < 0.001 |
| East coast | 452 (17.5) | 575 (22.2) | 595 (23.0) | 636 (24.6) | 643 (24.8) | |
| Central | 602 (23.3) | 997 (38.5) | 910 (35.2) | 868 (33.6) | 896 (34.6) | |
| West | 1,099 (42.5) | 542 (21.0) | 461 (17.8) | 439 (17.0) | 552 (21.3) | |
| Family household income[a], yuan | 5777.20 ± 6973.13 | 6060.39 ± 8012.01 | 6502.67 ± 7622.00 | 6278.35 ± 7192.21 | 6048.89 ± 7136.41 | 0.007 |
| Physical activity, MET-h/w | 161.61 ± 78.51 | 165.15 ± 92.30 | 179.85 ± 97.44 | 188.26 ± 100.74 | 199.15 ± 102.48 | < 0.001 |
| BMI, kg/m$^2$ | | | | | | |
| < 18.5 | 171 (6.6) | 189 (7.3) | 151 (5.8) | 115 (4.4) | 111 (4.3) | < 0.001 |
| 18.5 to 23.9 | 1,621 (62.6) | 1715 (66.3) | 1,699 (65.7) | 1726 (66.7) | 1732 (66.9) | |
| ≥ 24 | 796 (30.8) | 683 (26.4) | 737 (28.5) | 746 (28.8) | 745 (28.8) | |
| SBP, mmHg | 119.84 ± 16.15 | 120.28 ± 17.04 | 120.98 ± 16.38 | 120.98 ± 16.23 | 119.96 ± 16.41 | 0.023 |
| DBP, mmHg | 77.82 ± 10.04 | 77.78 ± 10.28 | 78.71 ± 10.13 | 78.52 ± 10.51 | 78.09 ± 9.96 | 0.002 |
| History of hypertension, % | 347 (13.4) | 126 (4.9) | 123 (4.8) | 104 (4.0) | 128 (4.9) | < 0.001 |
| History of diabetes, % | 95 (3.7) | 24 (0.9) | 21 (0.8) | 12 (0.5) | 18 (0.7) | < 0.001 |
| Smoking status | | | | | | |
| Never | 1941 (75.0) | 1962 (75.8) | 1868 (72.2) | 1723 (66.6) | 1,610 (62.2) | < 0.001 |
| Former | 49 (1.9) | 40 (1.5) | 31 (1.2) | 35 (1.4) | 48 (1.9) | |
| Current | 598 (23.1) | 585 (22.6) | 688 (26.6) | 829 (32.0) | 930 (35.9) | |
| Alcohol intake | | | | | | |
| Never | 1880 (72.6) | 1858 (71.8) | 1739 (67.2) | 1,670 (64.6) | 1,672 (64.6) | < 0.001 |
| < 3 times per week | 390 (15.1) | 415 (16.0) | 466 (18.0) | 499 (19.3) | 499 (19.3) | |
| ≥ 3 times per week | 318 (12.3) | 314 (12.1) | 382 (14.8) | 418 (16.2) | 417 (16.1) | |
| Dietary intake | | | | | | |
| Total energy, kcal/d | 2032.66 ± 631.08 | 2111.11 ± 567.66 | 2217.68 ± 563.96 | 2373.77 ± 610.89 | 2608.64 ± 668.76 | < 0.001 |
| Carbohydrate, % energy/d | 57.47 ± 13.69 | 58.54 ± 12.12 | 60.31 ± 11.81 | 61.33 ± 11.85 | 64.07 ± 11.38 | < 0.001 |
| Protein, % energy/d | 12.60 ± 2.96 | 12.47 ± 2.71 | 12.18 ± 2.64 | 12.11 ± 2.60 | 11.87 ± 2.38 | < 0.001 |
| Fat, % energy/d | 29.25 ± 12.81 | 28.33 ± 11.70 | 26.76 ± 11.13 | 25.81 ± 11.14 | 23.56 ± 11.07 | < 0.001 |
| Protein at breakfast, % breakfast energy[b] | 13.11 ± 9.67 | 12.04 ± 3.66 | 11.97 ± 3.22 | 11.57 ± 3.36 | 10.61 ± 3.97 | < 0.001 |
| Fat at breakfast, % breakfast energy | 12.60 ± 15.45 | 10.44 ± 8.76 | 11.21 ± 8.56 | 10.88 ± 8.70 | 10.93 ± 8.97 | < 0.001 |
| Carbohydrates at breakfast, % breakfast energy | 59.31 ± 31.86 | 77.57 ± 11.08 | 76.91 ± 10.23 | 77.70 ± 11.15 | 78.59 ± 11.04 | < 0.001 |
| SFA, g/d | 22.99 ± 15.05 | 23.78 ± 17.36 | 23.92 ± 18.46 | 25.01 ± 20.37 | 26.83 ± 23.12 | < 0.001 |
| Grains, g/d | 379.48 ± 172.11 | 406.49 ± 140.37 | 451.11 ± 151.34 | 493.58 ± 164.65 | 585.32 ± 211.61 | < 0.001 |
| Vegetables, g/d | 279.40 ± 152.26 | 300.70 ± 158.67 | 306.42 ± 160.03 | 319.22 ± 166.90 | 331.13 ± 169.36 | < 0.001 |
| Dietary fiber, g/d | 9.12 ± 5.89 | 9.06 ± 3.94 | 9.98 ± 4.31 | 10.70 ± 4.79 | 11.81 ± 6.81 | < 0.001 |

An ANOVA was used to test the differences in continuous variables across quintiles of energy intake at breakfast, and data were depicted by means ± SDs; a chi-square test was used for categorical variables, and data were depicted by frequencies (%). [a] Family household income was inflated to 2015. [b] refers to the percentage of breakfast energy intake from macronutrients at breakfast.

TABLE 2 Association between energy and percentage energy from macronutrients at breakfast and health outcomes.

| | Incidence (per 1,000 person-years; 95% CI) | | | | | Hazard ratio (95% CI) | | | | $p_{trend}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | Quintile 1 | Quintile 2 | Quintile 3 | Quintile 4 | Quintile 5 | Quintile 2 vs. Quintile 1 | Quintile 3 vs. Quintile 1 | Quintile 4 vs. Quintile 1 | Quintile 5 vs. Quintile 1 | |
| The energy at breakfast (kcal/d) | | | | | | | | | | |
| The range of value | < 268.2 | 268.2–359.0 | 359.0–443.3 | 443.3–562.6 | > 562.6 | | | | | |
| Major cardiovascular events | 5.6 | 3.7 | 3.3 | 2.6 | 3.1 | 0.95 | 0.91 | 0.71 | 0.86 | 0.1364 |
| | (4.6–6.6) | (3.0–4.4) | (2.6–4.0) | (2.0–3.2) | (2.4–3.8) | (0.72–1.26) | (0.68–1.22) | (0.52–0.98) | (0.62–1.20) | |
| Myocardial infarction | 1.8 | 1.9 | 1.7 | 1.2 | 1.1 | 1.51 | 1.44 | 1.03 | 0.95 | 0.506 |
| | (1.2–2.3) | (1.4–2.5) | (1.2–2.2) | (0.8–1.6) | (0.7–1.5) | (0.98–2.33) | (0.92–2.25) | (0.63–1.67) | (0.55–1.63) | |
| Stroke | 4.1 | 2 | 1.9 | 1.6 | 2.2 | 0.73 | 0.73 | 0.61 | 0.81 | 0.16 |
| | (3.2–4.9) | (1.5–2.6) | (1.4–2.5) | (1.1–2.1) | (1.6–2.8) | (0.51–1.04) | (0.51–1.06) | (0.41–0.91) | (0.55–1.21) | |
| Energy from carbohydrates (% breakfast energy) | | | | | | | | | | |
| The range of value | < 66.6 | 66.6–75.5 | 75.5–81.3 | 81.3–86.3 | > 86.3 | | | | | |
| Major cardiovascular events | 6.7 | 3.2 | 2.9 | 2.8 | 3.1 | 0.7 | 0.73 | 0.61 | 0.6 | < 0.0001 |
| | (5.5–7.8) | (2.5–3.9) | (2.3–3.6) | (2.2–3.4) | (2.5–3.8) | (0.52–0.94) | (0.54–0.99) | (0.45–0.83) | (0.44–0.81) | |
| Myocardial infarction | 2.6 | 1.6 | 1.4 | 1.1 | 1.2 | 0.92 | 0.87 | 0.65 | 0.69 | 0.0529 |
| | (1.9–3.3) | (1.1–2.1) | (0.9–1.8) | (0.7–1.5) | (0.8–1.6) | (0.60–1.40) | (0.56–1.35) | (0.40–1.04) | (0.43–1.11) | |
| Stroke | 4.5 | 1.8 | 1.7 | 1.9 | 2.2 | 0.59 | 0.66 | 0.62 | 0.58 | 0.0121 |
| | (3.6–5.4) | (1.2–2.3) | (1.2–2.2) | (1.4–2.5) | (1.6–2.7) | (0.40–0.87) | (0.45–0.97) | (0.43–0.89) | (0.40–0.86) | |
| Energy from protein (% breakfast energy) | | | | | | | | | | |
| The range of value | < 8.9 | 8.9–11.3 | 11.3–12.6 | 12.6–14.3 | > 14.3 | | | | | |
| Major cardiovascular events | 3 | 3.5 | 3.1 | 3.1 | 5.6 | 1.26 | 1.18 | 1.23 | 1.72 | 0.0024 |
| | (2.3–3.7) | (2.7–4.2) | (2.5–3.8) | (2.4–3.7) | (4.6–6.6) | (0.91–1.75) | (0.85–1.64) | (0.88–1.73) | (1.25–2.36) | |
| Myocardial infarction | 1.1 | 1.2 | 1.3 | 1.8 | 2.4 | 1.21 | 1.29 | 1.81 | 1.81 | 0.0058 |
| | (0.7–1.5) | (0.8–1.7) | (0.9–1.7) | (1.3–2.3) | (1.7–3.0) | (0.71–2.06) | (0.76–2.18) | (1.09–3.00) | (1.09–3.01) | |
| Stroke | 2.5 | 2.5 | 2.1 | 1.5 | 3.6 | 1.24 | 1.07 | 0.88 | 1.53 | 0.1606 |
| | (1.9–3.1) | (1.9–3.1) | (1.5–2.6) | (1.1–2.0) | (2.8–4.3) | (0.84–1.82) | (0.72–1.59) | (0.57–1.35) | (1.04–2.25) | |
| Energy from fat (% breakfast energy) | | | | | | | | | | |
| The range of value | < 3.2 | 3.2–6.3 | 6.3–10.7 | 10.7–17.8 | > 17.8 | | | | | |
| Major cardiovascular events | 3.6 | 3.5 | 2.5 | 2.9 | 5.9 | 1.41 | 1.02 | 1.14 | 1.74 | 0.007 |
| | (2.8–4.3) | (2.8–4.3) | (1.9–3.1) | (2.3–3.6) | (4.9–6.9) | (1.03–1.92) | (0.72–1.44) | (0.81–1.61) | (1.27–2.36) | |
| Myocardial infarction | 1.1 | 1.5 | 1.1 | 1.5 | 2.6 | 1.74 | 1.32 | 1.67 | 2.03 | 0.0168 |
| | (0.6–1.5) | (1.0–2.0) | (0.7–1.5) | (1.1–2.0) | (1.9–3.3) | (1.02–2.97) | (0.75–2.30) | (0.99–2.83) | (1.23–3.37) | |
| Stroke | 2.7 | 2.3 | 1.5 | 1.7 | 3.8 | 1.28 | 0.9 | 0.94 | 1.64 | 0.0732 |
| | (2.0–3.3) | (1.7–2.8) | (1.0–1.9) | (1.2–2.2) | (3.0–4.6) | (0.88–1.87) | (0.58–1.39) | (0.61–1.46) | (1.12–2.41) | |

Hazard ratios and 95% CIs have been adjusted for age, gender, urban or rural location, education level, income, physical activity, smoking status, alcohol intake, BMI, history of hypertension, diabetes, total intake of energy, SFA, grains, vegetables, and dietary fiber. The Cox frailty model was used with household identification as random intercepts. Major cardiovascular events include myocardial infarction and stroke.

without the presence of hypertension at baseline (Supplementary Figure 1). On the contrary, a percentage of energy from protein or fat intake was positively associated with risks of major cardiovascular events and new-onset stroke in participants without hypertension (Supplementary Figures 2, 3).

## 3.5. Sensitivity analysis

After additional adjustment for skipped breakfast, sensitivity analyses showed that the relationship among major cardiovascular events, myocardial infarction, new-onset stroke, and macronutrient intake remained consistent with the previous results, with a more significant $p_{trend}$ (Supplementary Table 3).

# 4. Discussion

Our study demonstrated that, based on this nationally representative sample of Chinese adults, adequate energy intake at breakfast might be associated with major cardiovascular events and stroke. Second, we also found that a relatively higher intake of

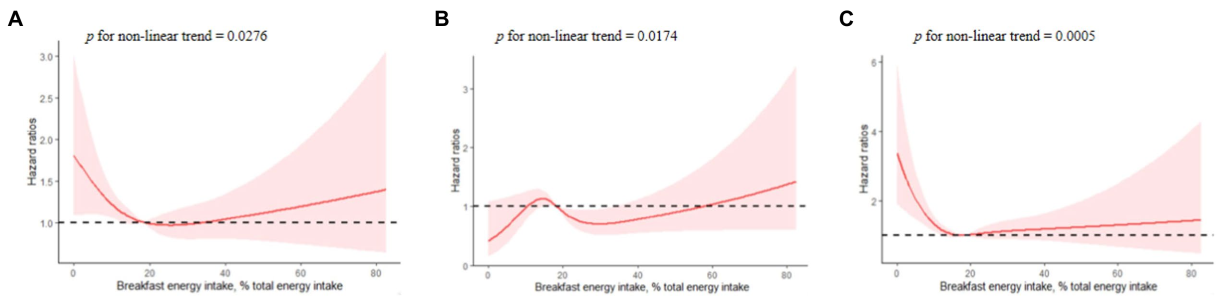


FIGURE 2
Multivariable-adjusted hazard ratios of association between breakfast energy intake and cardiovascular events [**(A)** major cardiovascular events; **(B)** myocardial infarction; **(C)** new-onset stroke]. A knot is located at the 5th, 35th, 65th, and 95$^{th}$ percentiles for energy intake at breakfast. The model was fully adjusted for age, gender, urban or rural location, education level, income, physical activity, smoking status, alcohol intake, BMI, history of hypertension, diabetes, total intake of energy, SFA, grains, vegetables, and dietary fiber, with household identification as random intercepts.

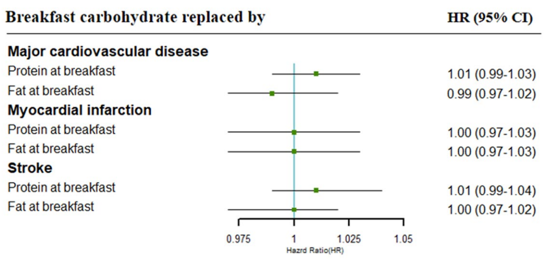


FIGURE 3
Cardiovascular risk associated with isocaloric (5% of energy) replacement of carbohydrates with other specific macronutrients. Adjustments included age, gender, urban or rural location, education level, income, physical activity, smoking status, alcohol intake, BMI, history of hypertension, diabetes, energy intake at breakfast, SFA, grains, vegetables, and dietary fiber.

carbohydrates at breakfast was associated with a lower risk of cardiovascular disease. By contrast, a higher intake of protein and fat at breakfast was positively associated with the risk of cardiovascular disease. The link between the proportion of breakfast carbohydrates, protein, and fat intake and reduced cardiovascular events was stronger in participants without hypertension.

Previous studies have focused on the relationship between breakfast consumption and cardiovascular and metabolic disorders. The higher the breakfast energy intake, the lower the risk of CVD, as demonstrated in Western countries (10, 17). We also found that more energy intakes at breakfast may reduce the risk of stroke in men, which was consistent with the CIRCS study in the Japanese population (23). An animal experiment showed that high energy intake at breakfast reversed the expression of disrupted clock genes and had beneficial effects on lipid control (44). This conclusion is largely based on selective subgroup comparisons between skipping/irregular breakfast and regular breakfast eating, despite a recent finding that the benefits of breakfast intake may be related to meal quality rather than skipping/eating breakfast, due to unknown confounding factors (14). In the current study, an important finding was that appropriate intake of energy at breakfast was positively associated with the risk of major CVD and stroke, and the associations were independent of skipping breakfast. But there was no significant relationship of breakfast energy intake with MI. In conducting independent analyses of breakfast energy intake and MI, the effect of stroke as a factor may have been overlooked as to a few samples that experienced a stroke before MI. This may be due to changes in lifestyle and eating habits following a stroke, which in turn affects the relationship between breakfast energy intake and MI.

As a source of carbohydrates in the body, carbohydrates are one of the essential energy-yielding nutrients for the human body to sustain life activities. The "Dietary Guidelines for Chinese Residents (2022)" recommends that the energy provided by carbohydrates in the daily diet account for 50 to 60% of the total energy, but does not mention the proportion of energy intake at breakfast. In this study, we concluded that energy from carbohydrates at breakfast accounted for 76.4% of breakfast energy intake, which was similar to what was found in a previous study (45). The "carbohydrate-insulin model" has been proposed, arguing that a high intake of carbohydrates leads to endocrine dysregulation marked by hyperinsulinemia, driving energy allocation and increased energy storage in adipose tissue (46, 47). Although high intake of carbohydrates was linked to cardiovascular risk factors, it was unknown whether carbohydrate intake at breakfast affects the body. Our research revealed a very interesting conclusion that a higher intake of breakfast carbohydrates was associated with a reduced risk of CVD, especially in female population, which was contrary to other studies (48–50). One reason is possibly that the energy provided by breakfast carbohydrate intake is derived from the calculation of breakfast energy intake in our study rather than total daily energy intake in other studies. This suggests that a higher intake of carbohydrates, compared to protein and fat at breakfast, was beneficial for preventing cardiovascular events. Interestingly, a systematic review has indicated that carbohydrate quality may be more important than quantity when assessing the relationship between carbohydrate intake and cardiometabolic outcomes (51). Therefore, future studies should further evaluate the association between food sources of breakfast carbohydrates and CVD.

In the current study, there were no associations between source-specific protein intake (e.g., animal versus plant sources) and a range of CVD risk. Breakfast protein intake was positively associated with CVD incidence, which was inconsistent with many previous studies (52, 53). By the comparison of two systematic reviews (26, 54), we found differences in the associations between the exposure variables of meat as a food group and protein as a nutrient and CVD. In addition, to the best of our knowledge, dietary patterns

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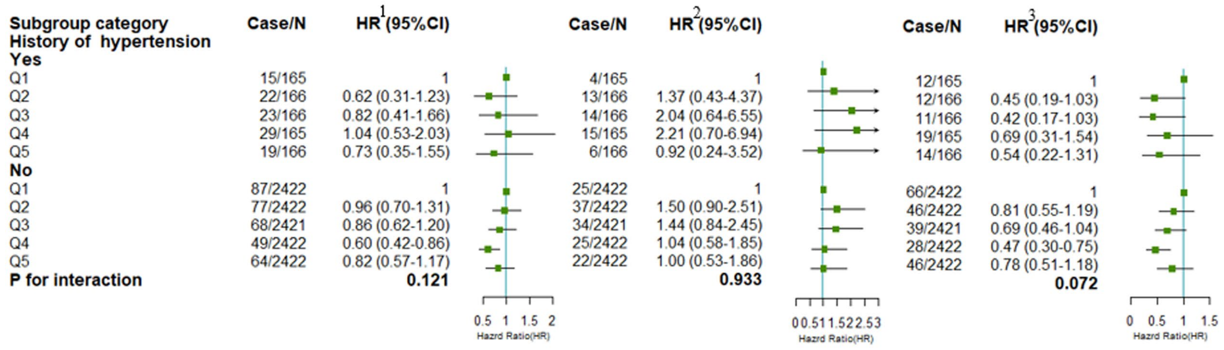


FIGURE 4
Stratified analyses by the history of hypertension at baseline of the association between breakfast energy intake and health outcomes. Adjustments included age, gender, urban or rural location, education level, income, physical activity, smoking status, alcohol intake, BMI, history of diabetes, total intake of energy, SFA, grains, vegetables, and dietary fiber, if not stratified. [1] refers to major cardiovascular events; [2] refers to myocardial infarction; [3] refers to new-onset stroke.

have been undergoing rapid transitions in China over the past two decades. The amount of consuming meat continued to increase dramatically, with livestock and poultry intake far above the recommendations (55). Moreover, adults eating away-from-home meals are more likely to consume processed meat, which may contribute to the burden of CVD. Considering this may be the reason for the inconsistent results of our study and other studies based on the non-Chinese population.

For breakfast fat intake, we found a positive correlation between energy intake from fat and the risk of major cardiovascular events, myocardial infarction, and stroke. Previous studies have demonstrated the effect of breakfast fat intake on cardiovascular health. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-monocyte colony-stimulating factor (GM-CSF) were elevated after a high-fat breakfast, which has implications for adipose tissue inflammation and the risk of developing atherosclerosis (56). Additionally, a crossover clinical trial has suggested that dietary intake of unsaturated fatty acids at breakfast may induce an anti-inflammatory response, in turn affecting cardiometabolic risk (57). Inconsistent with our findings, the replacement of saturated fatty acids with unsaturated fatty acids may reduce cardiovascular risk (21, 22). However, a recent systematic review showed a lack of rigorous evidence for limiting the consumption of saturated fatty acids or replacing saturated fatty acids with polyunsaturated fatty acids (58). There is much evidence that eating a high-fat breakfast has adverse effects on cardiovascular health, but further well-conducted cohort studies are needed to identify the relationship between types of fatty acids and cardiovascular health.

To our knowledge, this is the first longitudinal study to use a health and nutrition survey database in a large sample population from nine provinces in China. It is noteworthy that our study yielded somewhat different results from previous studies by a risk assessment of the association between breakfast energy and macronutrient intake and cardiovascular disease. However, our study had some limitations. First, self-reported disease diagnosis was subjective and potentially associated with recall bias. Second, although the adjustment for socioeconomic and lifestyle factors and intakes of main food groups, unknown confounding factors that we did not account for are possible. Third, the 24-h meal recall method generally cannot accurately assess daily dietary intake.

## 5. Conclusion

Breakfast is part of the daily diet, and eating breakfast every day is also a healthy lifestyle advocated by the World Health Organization. Chinese dietary nutritional guidelines also point out that a reasonable diet, part of which is eating breakfast regularly, can reduce the risk of cardiovascular disease. As the first meal of the day, breakfast is very important for dietary nutrition intake. In conclusion, a higher intake of carbohydrates, and a relatively lower intake of protein and fat at breakfast may contribute to cardiovascular health on the basis of maintaining a moderate energy intake. This information is of importance in providing nutritional recommendations, especially a reasonable combination of breakfast nutrition for the public. Therefore, in the perspective of the future, we need to further explore the impact of macronutrient quality on cardiovascular disease in addition to macronutrient quantity.

## 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 in the article/Supplementary material.

## Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Committees of the University of North Carolina at Chapel Hill and the National Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

XD and RY made substantial contributions to the conception and design of the study. XD was a major contributor in writing the first draft of the manuscript. Data collection and statistical analysis were performed by MM and SK with assistance from XD. RY, JZ, and XT reviewed and commented on the first draft. 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.2023.1093561/full#supplementary-material

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# Strategies for reducing meat consumption within college and university settings: A systematic review and meta-analysis

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**Introduction:** Despite the considerable public and planetary health benefits associated with reducing the amount of meat consumed in high-income countries, there is a limited empirical understanding of how these voluntary changes in food choice can be effectively facilitated across different settings. While prior reviews have given us broad insights into the varying capacities of behavior change strategies to promote meaningful reductions in meat consumption, none have compared how they perform relative to each other within a uniform dining context.

**Methods:** To address this gap in the literature, we synthesized the available research on university-implemented meat reduction interventions and examined the variations in the success rates and effect estimates associated with each of the three approaches identified in our systematic review.

**Results:** From our analyses of the 31 studies that met our criteria for inclusion ($n = 31$), we found that most were successful in reducing the amount of meat consumed within university settings. Moreover, independent of the number of individual strategies being used, multimodal interventions were found to be more reliable and effective in facilitating these changes in food choice than interventions targeting the choice architecture of the retail environment or conscious decision-making processes alone.

**Discussion:** In addition to demonstrating the overall value of behavior change initiatives in advancing more sustainable dining practices on college and university campuses, this study lends further insights into the merits and mechanics underlying strategically integrated approaches to dietary change. Further investigations exploring the persistence and generalizability of these effects and intervention design principles are needed.

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KEYWORDS

**dietary change, health promotion, systematic review, universities and higher education institutions, behavioral interventions, meat reduction, meta-analysis, sustainable nutrition**

# 1. Introduction

Dietary change has been cited as a necessary measure for achieving a variety of international sustainability targets (Springmann et al., 2018; Clark et al., 2020; Chen et al., 2022). In particular, there has been an emphasis on the co-benefits of reducing excess meat consumption among adolescents and adults living in high-income countries (HICs) (Yip et al., 2013; Aleksandrowicz et al., 2016; Springmann et al., 2016), specifically with regard to the advantages these population-level shifts would present for human health, climate change, and the global ecology (Godfray et al., 2018; Willett et al., 2019; Parlasca and Qaim, 2022).

Programs aimed at limiting the sale and consumption of meat have therefore become an important object of interest among food systems researchers (De Boer et al., 2014; Garnett et al., 2015; Wellesley et al., 2015; Jiang et al., 2020; Rust et al., 2020), with evidence to suggest that actualizing these transitions within HICs could reduce agricultural emissions, water use, and land appropriation by more than half (Aleksandrowicz et al., 2016; Sun et al., 2022), all while avoiding millions of diet-related deaths each year (Wang et al., 2016; Willett et al., 2019; Zhang et al., 2021).

Due to pervasive public resistance to programs restricting individual choice (Sievert et al., 2020; Ewald et al., 2021; Pechey et al., 2022), much of this research has focused on the value of behavior change strategies in promoting voluntary shifts in food choice (Bianchi et al., 2018a,b; Graça et al., 2019). Within the context of meat reduction interventions, common strategies include those that (1) modify the presentation and arrangement of items on menus, (2) add to the existing set of meal options, (3) manipulate the layouts of dining areas, (4) utilize promotional messaging, and (5) introduce pricing incentives.

Each of these strategies leverage different behavior change principles to motivate individuals toward more sustainable food choices without actively limiting the options available to them. Dual-process accounts of conditional reasoning (Evans, 2011; Kahneman, 2011) provide us with a useful framework for classifying these differences, with some strategies targeting more implicit decision-making processes and others focusing instead on the more deliberate aspects of cognition.

Using this operating principle, we can group the existing set of meat reduction interventions into three categories (referred to as "approaches" hereafter) based on the cognitive systems being targeted by their individual strategies. More specifically, we specify between (1) interventions that target the choice architecture of the retail environment (i.e., interventions that modify the presentation and arrangement of items on menus, add to the existing set of meal options, and/or manipulate the layouts of dining areas), (2) interventions that target conscious decision-making processes (i.e., interventions that utilize promotional messaging and/or introduce pricing incentives), and (3) interventions that target both systems simultaneously (i.e., interventions that involve at least two strategies corresponding to each of the initial two approaches; referred to as "multimodal interventions" hereafter) (see Figure 1).

While prior reviews have given us broad insights into the value of dietary change interventions in shifting attitudes (Hartmann and Siegrist, 2017; Sanchez-Sabate and Sabaté, 2019; Valli et al., 2019) and behaviors (Bianchi et al., 2018a,b; Harguess et al., 2020; Kwasny et al., 2022) related to meat consumption, none have compared how these approaches perform relative to each other within a uniform dining context—information that could meaningfully inform the underlying theory and design of setting-specific policies and interventions. In the health promotion literature, settings-based evaluation techniques are frequently used to identify overlapping traits between high-performing interventions, allowing researchers to isolate the strategic components that are most valuably contributing to the behavioral changes being observed with that context (Whitelaw et al., 2001; Dooris, 2009; Bloch et al., 2014).

Due to the high mitigation potential associated with lowering the resource requirements tied to institutional foodservice operations (Jones et al., 2019; Bull et al., 2022; Sherry and Tivona, 2022) and the developing interest in improving the sustainability of college and university environments (Leal Filho et al., 2018; Amaral et al., 2020; Ruiz-Mallén and Heras, 2020), we elected to apply these techniques toward the subset of meat reduction interventions that have been implemented within higher education institutions (HEIs) (referred to as "university settings" hereafter). In particular, we wanted to leverage this existing evidence base to (1) determine whether meat reduction interventions have been successful in reducing the amount of meat consumed within university settings and (2) identify which of the three investigated approaches has generated the most favorable dietary change outcomes.

To accomplish this, we synthesized the existing research on university meat reduction and examined the variations in the success rates and effect estimates between interventions targeting the choice architecture of the retail environment, interventions targeting conscious decision-making processes, and multimodal interventions. Based on these analyses, we found that the majority of included interventions were successful in reducing the amount of meat consumed within university settings, and that multimodal interventions were more reliable and effective in facilitating these changes than either of the remaining two approaches, regardless of the number of individual strategies being used. Further insights regarding the state of the literature and its possible future directions are also provided.

# 2. Materials and methods

This paper has been presented in accordance with the guidelines stipulated by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (see Supplementary Table 1). For further information on our methodologies, a more detailed version of this previously registered protocol can be viewed alongside a record of its revisions at https://osf.io/zhg5b/.

## 2.1. Search strategy

To identify the relevant literature published between January 1, 2000, and June 3, 2021, we consulted seven electronic databases: ERIC, PsycINFO, PubAg, PubMed, SCOPUS, Sociological Abstracts, and Web of Science. For each database, we used a combination of keywords and controlled vocabulary operationalizing our three principal search concepts: behavior change, meat consumption, and college and university settings.

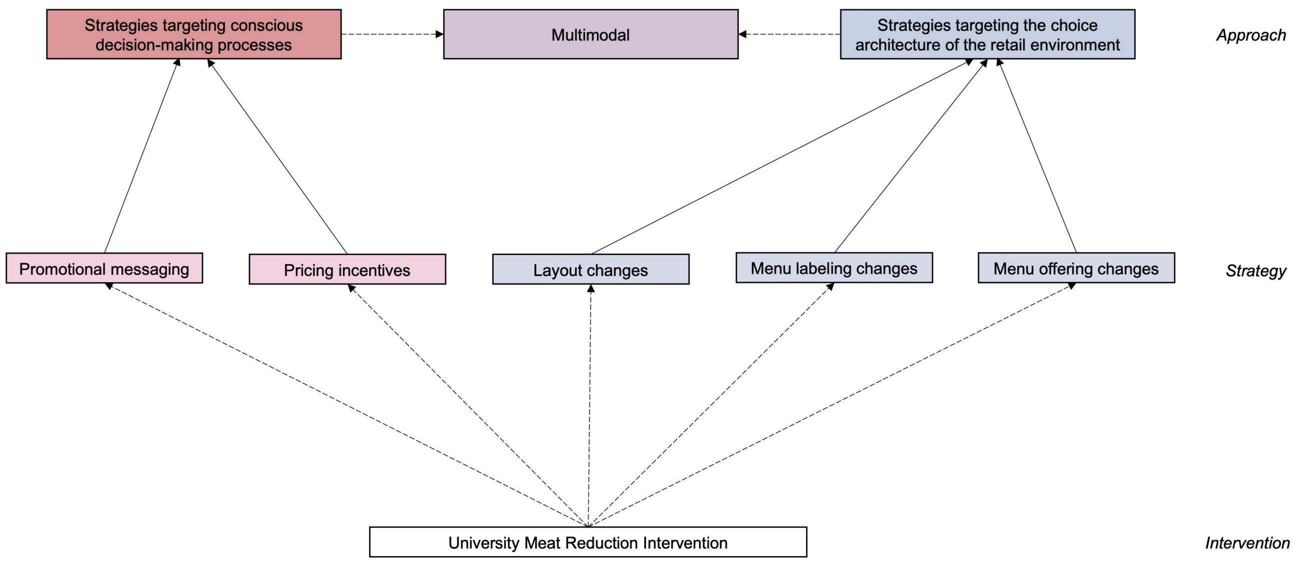


FIGURE 1
Flow diagram outlining the organizing principles of our intervention classification scheme. Interventions are considered "multimodal" if they involved two or more strategies corresponding to each of the remaining two approach styles. Therefore, at minimum, multimodal interventions must include at least one strategy targeting the choice architecture of the retail environment and at least one strategy targeting conscious decision-making processes.

To develop these searches, we generated an initial base strategy for the PubMed database using the Yale Medical Subject Heading (MeSH) Analyzer to create a list of terms represented across 11 previously identified seed articles. After filtering these terms based on their relevance to our search concepts, we translated the resulting base strategy across the remaining six databases. These searches are provided in full in Supplementary Table 2.

## 2.2. Selection criteria

Prior to implementing our search strategy, we developed a set of selection criteria based on our research objectives and pre-existing knowledge of the literature. These specifications were designed to parameterize our analyses by qualifying eligible studies based on (1) the populations that were sampled, (2) the settings that were investigated, (3) the outcome variables that were assessed, and (4) the evaluation methods that were used. We elaborate on these criteria in greater detail below.

### 2.2.1. Population

Studies were required to sample members of the hosting university's student body. To allow exploration into the effects of interventions on the broader university environment, we elected to retain instances where members of the faculty and staff were sampled alongside university students.

### 2.2.2. Setting

Studies were required to report on interventions that had been conducted naturalistically within physical university dining environments. Records documenting laboratory-based, artificial choice experiments were excluded.

### 2.2.3. Evaluation method

Studies were required to evaluate the effects of a campaign, program, or initiative in at least one of two ways: (1) by comparing behavioral measures before and after the implementation of an intervention or (2) by making comparisons between treatment and control groups. Studies utilizing cross-sectional data were excluded.

### 2.2.4. Outcome variables

Studies were required to use at least one of the following three measures to evaluate the effect of the intervention on food choice: (1) intended changes in meat consumption, (2) self-reported changes in meat consumption, and (3) observed changes in meat consumption. Studies using alternate measures of food choice were excluded.

## 2.3. Screening procedure

After importing the records into a citation management system (Clarivate, 2020), we documented the total volume of search results before removing duplicate entries. Non-duplicate records were then transferred to an online web-based program for screening (Veritas Health Innovation, 2021).

Our screening procedure was conducted successively by two independent reviewers, with a third designated to settle arising conflicts in voting. The initial stage of title-and-abstract screening

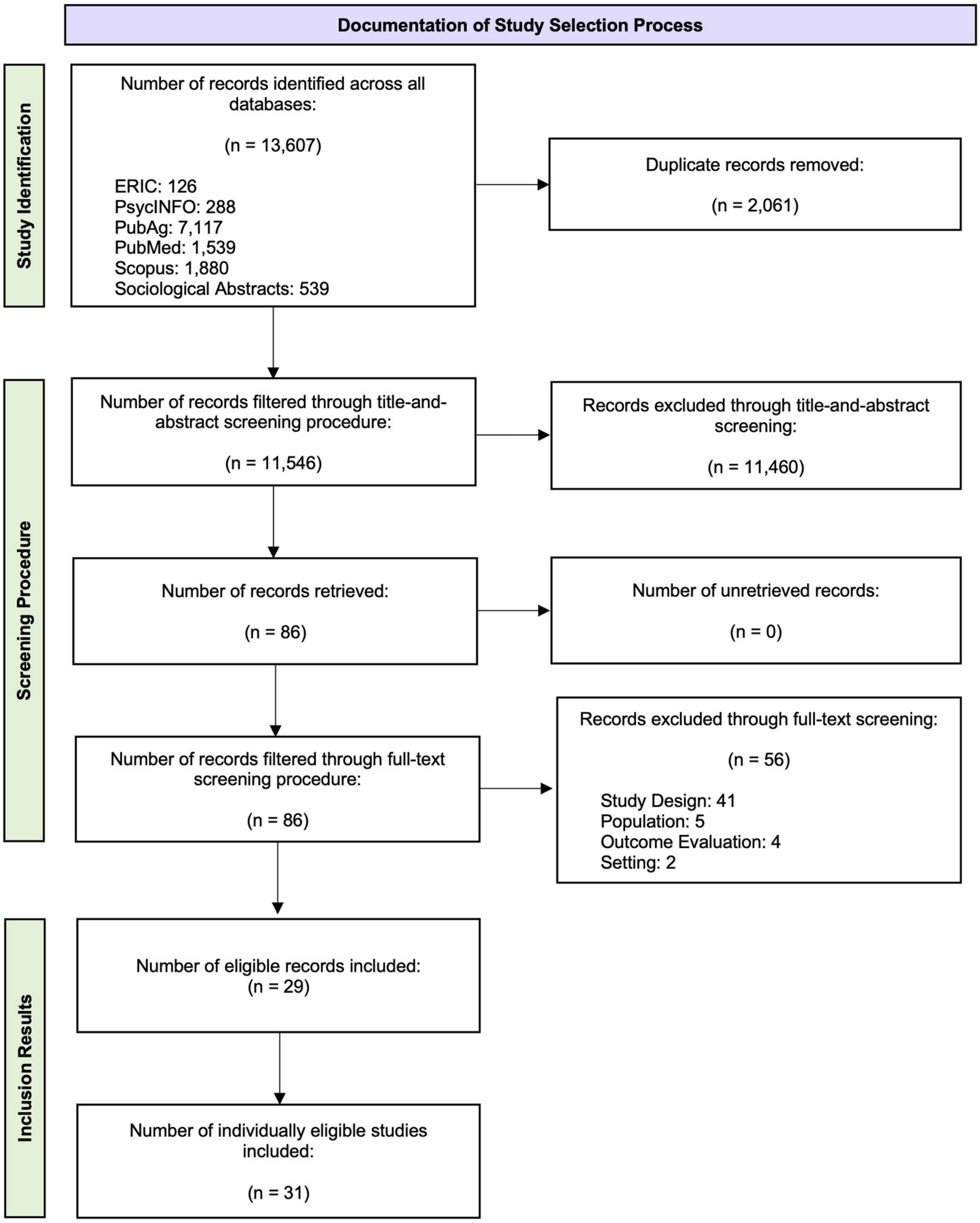


FIGURE 2
Adapted PRISMA 2020 flow diagram outlining the stepwise results of our study selection process. There were seven instances where a third independent reviewer was needed to settle arising disputes in voting. Each of these instances emerged during the initial stage of title-and-abstract screening and were ultimately resolved *via* group consensus.

was used to sort non-duplicate records according to their potential relevance, with all decisions being based around whether the readable contents met any of our four exclusion criteria. By contrast, during the final stage of full-text review, records were only included if the readable contents met all four of our inclusion criteria (see Figure 2).

## 2.4. Data extraction

For each included record, data on the article's author(s), title, publication year, and implementation year were collected alongside information describing the university and country in which the intervention was implemented. Using the classification scheme described in Figure 1, we typified included interventions based on the strategies they used and the larger approach category they fell under. Information on their evaluation methods, outcome measures, and targeted meat types were also extracted.

To standardize how significance was determined across studies, we also collected the relevant data used to interpret the intervention's effect on meat consumption. This involved extracting study-level information on mean outcomes, group sample sizes, and error. All extracted variables were exported to and compiled in Microsoft Excel (see Table 1) (Microsoft Corporation, 2018).

## 2.5. Quality assessment

We used the Evidence Project's Risk of Bias Tool to establish a minimum standard for our analyses (Viswanathan et al., 2018; Kennedy et al., 2019), such that each included study needed to meet at least one of the eight specified criteria for inclusion. This was done to ensure that studies were of sufficient rigor and appropriately accounted for common sources of bias.

Studies were therefore required to satisfy at least one of the following eight criteria: (1) monitor a cohort over time, (2) involve the use of a control or comparison group, (3) use pre-post intervention data, (4) use random assignment, (5) use random selection, (6) report a rate of attrition below 20 percent, (7) demonstrate equivalency across sociodemographic variables, or (8) demonstrate equivalency across outcome variable of interest (Kennedy et al., 2019).

## 2.6. Main analyses

Our main analyses were divided into three parts, with the first evaluating differences in the success rates across interventions, the second comparing mean differences in their effect estimates, and the third examining changes in performance over time.

### 2.6.1. Success rates

As a function of both the direction and significance of an intervention's effect on meat consumption, we used success rates to compare the capacities of the three investigated approaches to facilitate changes in food choice. These values were calculated by dividing the number of interventions reporting significant reductions in meat consumption within an approach category by the total number of interventions that used that particular approach.

To standardize how significance was determined across studies, we used a consistent set of methods to internally calculate changes in behavior resulting from the intervention. For studies inferring change based on the differences in the sample means reported between control and treatment conditions, we used two-tailed *t*-tests, and for those inferring change based on proportional differences in sales, we used chi-square tests. In both cases, we set an alpha of 0.05 and depicted these results visually using Boon and Thomson (2021) revised methods for visualizing patterns, which leverage the "<>," "∧," and "∨" symbols to represent neutral, positive, and negative effects, respectively.

### 2.6.2. Effect estimates

In addition to using success rates to understand the relative frequency with which interventions significantly reduced the amount of meat consumed within university settings, we also calculated the associated odds ratios to evaluate how the magnitudes of these effects varied across approaches. For the studies using mean differences to form these comparisons, we calculated these effect estimates using the means and standard deviations of the control and interventions groups, and for those using differences in sales proportions, effect estimates were calculated using the frequency distributions derived from the sample sizes and proportional shares of control and intervention groups.

To perform these calculations, we used the Practical Meta-Analysis Calculator from Lipsey and Wilson (2001) and later verified these initial results using the "effectsize" (Ben-Shachar et al., 2020) and "metafor" (Schwarzer, 2022) packages within RStudio (RStudio Team, 2021).

### 2.6.3. Time series

Prior research has indicated that there has been increasing concern and awareness surrounding how diets influence different processes related to global environmental change (Macidarmid et al., 2016; Jürkenbeck et al., 2021). To determine whether this phenomenon has contributed to differences in the performance of interventions over time, we used Sturge's rule to construct equal-sized time intervals and compared the success rates and effect estimates of interventions conducted between 2001 and 2007, 2008 and 2014, and 2015 and 2021.

# 3. Results

## 3.1. Study selection

Of the 11,546 unique records screened for their relevance and eligibility, a total of 29 research articles documenting 31 independently eligible studies met the necessary criteria for inclusion (see Figure 2).

## 3.2. Study characteristics

### 3.2.1. Time and place

The 31 studies included in our analyses were all conducted and published between 2001 and 2021, with each cumulative frequency distribution increasing exponentially over time ($R^2 = 0.97$, $R^2 = 0.96$) (see Figure 3A). The studies were conducted at a total of 33 different intervention sites spanning 24 colleges and universities, nine countries, and two continents (see Figure 3B). More than half were conducted in the United States (51.5%) while the remaining 16 were implemented at colleges and universities in England (16.1%),

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TABLE 1 Summary table outlining the study-level characteristics associated with each of the 31 interventions included in our analyses.

| Study (*n* = 31) | Article title | Country | Year conducted | Method of evaluation | Approach category | Strategies implemented | Reported outcome variable | Targeted meat types | Direction of effect | Odds ratio (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Andersson and Nelander (2021) | Nudge the lunch: A field experiment testing menu-primacy effects on lunch choices | Sweden | 2019 | Between-subjects comparisons | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Observed changes in meal purchasing behaviors | All meat | ^ | 2.78 (1.00, 7.75) |
| Brunner et al. (2018) | Carbon label at a university restaurant: Label implementation and evaluation | Sweden | 2016 | Pre-post | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Observed changes in meal purchasing behaviors | Ruminant meat | ^ | 1.09 (1.01, 1.18) |
| Campbell-Arvai (2011) | Motivating pro-environmental food choices: The role of value orientation, information provision, and a default behavioral intervention | USA | 2010 | Between-subjects comparisons | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Intentions to change meal purchasing behaviors | All meat | ^ | 12.19 (8.90, 16.68) |
| Carfora et al. (2019) | How to reduce red and processed meat consumption by daily text messages targeting environment or health benefits | Italy | 2018 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | Red and processed meat | ^ | 2.59 (1.35, 4.97) |
| Carfora et al. (2016) | Randomized controlled trial of a text messaging intervention for reducing processed meat consumption: The mediating roles of anticipated regret and intention | Italy | 2016 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | Processed meat | ^ | 3.49 (1.75, 6.96) |

*(Continued)*

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TABLE 1 (Continued)

| Study ($n$ = 31) | Article title | Country | Year conducted | Method of evaluation | Approach category | Strategies implemented | Reported outcome variable | Targeted meat types | Direction of effect | Odds ratio (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Carfora et al. (2017) | Correlational study and randomized controlled trial for understanding and changing red meat consumption: The role of identities | Italy | 2015 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | Red meat | ^ | 3.73 (2.29, 6.06) |
| Cerezo-Prieto and Frutos-Esteban (2021) | Toward healthy routes: Effects of nutritional labels on eating behaviors in a university cafeteria | Spain | 2020 | Pre-post | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Observed changes in meal purchasing behaviors | Red meat | ^ | 1.71 (1.37, 2.12) |
| Dissen and Crowell (2020) | We are what we eat: Assessing the use of a documentary film as an educational tool to change students' nutritional attitudes and behaviors | USA | 2019 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | Beef, pork, poultry | ^ | 3.20 (1.68, 6.08) |
| Garnett et al. (2021) | Price of change: Does a small alteration to the price of meat and vegetarian options affect their sales | England | 2018 | Between-subjects comparisons | Interventions targeting conscious decision-making processes | (1) Financial incentive | Observed changes in meal purchasing behaviors | All meat | <> | 1.07 (1.01, 1.14) |
| Garnett et al. (2020) | Order of meals at the counter and distance between options affect student cafeteria vegetarian sales | England | 2017 | Between-subjects comparisons | Interventions targeting aspects of the built environment | (1) Change in dining area layout | Observed changes in meal purchasing behaviors | All meat | <> | 0.82 (0.78, 0.88) |
| Garnett et al. (2020) | Order of meals at the counter and distance between options affect student cafeteria vegetarian sales | England | 2018 | Between-subjects comparisons | Interventions targeting aspects of the built environment | (1) Change in dining area layout | Observed changes in meal purchasing behaviors | All meat | ^ | 3.64 (3.51, 3.77) |
| Garnett et al. (2019) | Impact of increasing vegetarian availability on meal selection and sales in cafeterias | England | 2017 | Pre-post | Interventions targeting aspects of the built environment | (1) Change in menu offerings | Observed changes in meal purchasing behaviors | All meat | ^ | 1.52 (1.40, 1.64) |

(Continued)

TABLE 1 (Continued)

| Study (*n* = 31) | Article title | Country | Year conducted | Method of evaluation | Approach category | Strategies implemented | Reported outcome variable | Targeted meat types | Direction of effect | Odds ratio (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Hormes et al. (2013) | Reading a book can change your mind, but only some changes last for a year: Food attitude changes in readers of The Omnivore's Dilemma | USA | 2007 | Between-subjects comparisons | Interventions targeting conscious decision-making processes | (1) Informational messaging | Intentions to change meal purchasing behaviors | All meat | ^ | 1.75 (1.30, 2.35) |
| Jalil et al. (2020) | Eating to save the planet: Evidence from a randomized controlled trial using individual-level food purchase data | USA | 2019 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Observed changes in meal purchasing behaviors | All meat | <> | 1.57 (0.97, 2.56) |
| Jay et al. (2019) | Reduction of the carbon footprint of college freshman diets after a food-based environmental science course | USA | 2017 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | Ruminant meat | <> | 1.71 (0.97, 3.00) |
| Kurz (2018) | Nudging to reduce meat consumption: Immediate and persistent effects of an intervention at a university restaurant | Sweden | 2015 | Both | Interventions targeting aspects of the built environment | (1) Change in menu presentation<br>(2) Change in dining area layout | Observed changes in meal purchasing behaviors | All meat | <> | 1.44 (0.70, 2.99) |
| Larner et al. (2021) | Reaction to a low-carbon footprint food logo and other sustainable diet promotions in a UK University's Student Union "Living Lab" | England | 2019 | Pre-post | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation<br>(3) Financial incentive | Observed changes in meal purchasing behaviors | Ruminant meat | ^ | 1.41 (1.36, 1.46) |

TABLE 1 (Continued)

| Study ($n = 31$) | Article title | Country | Year conducted | Method of evaluation | Approach category | Strategies implemented | Reported outcome variable | Targeted meat types | Direction of effect | Odds ratio (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Malan (2020) | Swap the meat, save the planet: A community-based participatory approach to promoting healthy, sustainable food in a university setting | USA | 2019 | Both | Multimodal | (1) Informational messaging<br>(2) Change in menu offerings | Observed changes in meal purchasing behaviors | All meat | ^ | 1.39 (1.34, 1.44) |
| Malan et al. (2020) | Impact of a scalable, multi-campus "Foodprint" seminar on college students' dietary intake and dietary carbon footprint | USA | 2018 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | All meat | <> | 1.41 (0.83, 2.41) |
| McClain et al. (2013) | Incorporating prototyping and iteration into intervention development: A case study of a dining hall-based intervention | USA | 2011 | Both | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Self-reported changes in meal purchasing behaviors | Red and processed meat | ^ | 1.50 (0.94, 2.40) |
| McDonough (2012) | Modifying students' intentions to eat sustainably | Canada | 2010 | Pre-post | Interventions targeting conscious decision-making processes | (1) Informational messaging | Intentions to change meal purchasing behaviors | All meat | <> | 1.53 (0.83, 2.82) |
| Michels et al. (2008) | A study of the importance of education and cost incentives on individual food choices at the Harvard School of Public Health cafeteria | USA | 2001 | Pre-post | Interventions targeting conscious decision-making processes | (1) Informational messaging<br>(2) Financial incentive | Observed changes in meal purchasing behaviors | Red and processed meat | ^ | 2.82 (2.57, 3.10) |

TABLE 1 (Continued)

| Study (*n* = 31) | Article title | Country | Year conducted | Method of evaluation | Approach category | Strategies implemented | Reported outcome variable | Targeted meat types | Direction of effect | Odds ratio (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Nash (2014) | The green eating project: Web-based intervention to promote environmentally conscious eating behaviors | USA | 2013 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Intentions to change meal purchasing behaviors | All meat | <> | 1.33 (0.92, 1.91) |
| Papadaki and Scott (2005) | The Mediterranean Eating in Scotland Experience project: Evaluation of an internet-based intervention promoting the Mediterranean diet | Scotland | 2003 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | All meat | <> | 0.61 (0.23, 1.58) |
| Piester et al. (2020) | "I'll try the veggie burger": Increasing purchases of sustainable foods with information about sustainability and taste | USA | 2019 | Between-subjects comparisons | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Observed changes in meal purchasing behaviors | All meat | ^ | 1.87 (1.08, 3.24) |
| Piester et al. (2020) | "I'll try the veggie burger": Increasing purchases of sustainable foods with information about sustainability and taste | USA | 2019 | Between-subjects comparisons | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Observed changes in meal purchasing behaviors | All meat | ^ | 2.13 (1.47, 3.09) |

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TABLE 1 (Continued)

| Study ($n = 31$) | Article title | Country | Year conducted | Method of evaluation | Approach category | Strategies implemented | Reported outcome variable | Targeted meat types | Direction of effect | Odds ratio (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ring et al. (2019) | Cooking up health: A novel culinary medicine and service-learning elective for health professional students | USA | 2017 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | All meat | ^ | 17.97 (9.60, 33.63) |
| Schwitzgebel et al. (2020) | Do ethics classes influence student behavior? Case study: Teaching the ethics of eating meat | USA | 2017 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Observed changes in meal purchasing behaviors | All meat | ^ | 1.27 (1.16, 1.40) |
| Slapø and Karevold (2019) | Simple eco-labels to nudge customers toward the most environmentally friendly warm dishes: An empirical study in a cafeteria setting | Norway | 2018 | Pre-post | Multimodal | (1) Informational messaging<br>(2) Change in menu presentation | Observed changes in meal purchasing behaviors | All meat | ^ | 2.70 (1.04, 7.00) |
| Turnwald and Crum (2019) | Smart food policy for healthy food labeling: Leading with taste, not healthiness, to shift consumption and enjoyment of healthy foods | USA | 2017 | Between-subjects comparisons | Interventions targeting aspects of the built environment | (1) Change in menu presentation | Observed changes in meal purchasing behaviors | All meat | <> | 1.68 (1.46, 1.92) |
| Wolstenholme et al. (2020) | Two birds, one stone: The effectiveness of health and environmental messages to reduce meat consumption and encourage pro-environmental behavioral spillover | Wales | 2020 | Both | Interventions targeting conscious decision-making processes | (1) Informational messaging | Self-reported changes in meal purchasing behaviors | Red and processed meat | ^ | 5.21 (2.66, 10.20) |

The “^,” “<>,” and “∨” symbols were adapted from Boon and Thomson’s (2021) revised methods for visualizing patterns and used to represent whether the intervention had a positive, neutral, or negative effect, respectively, on the outcome of interest.

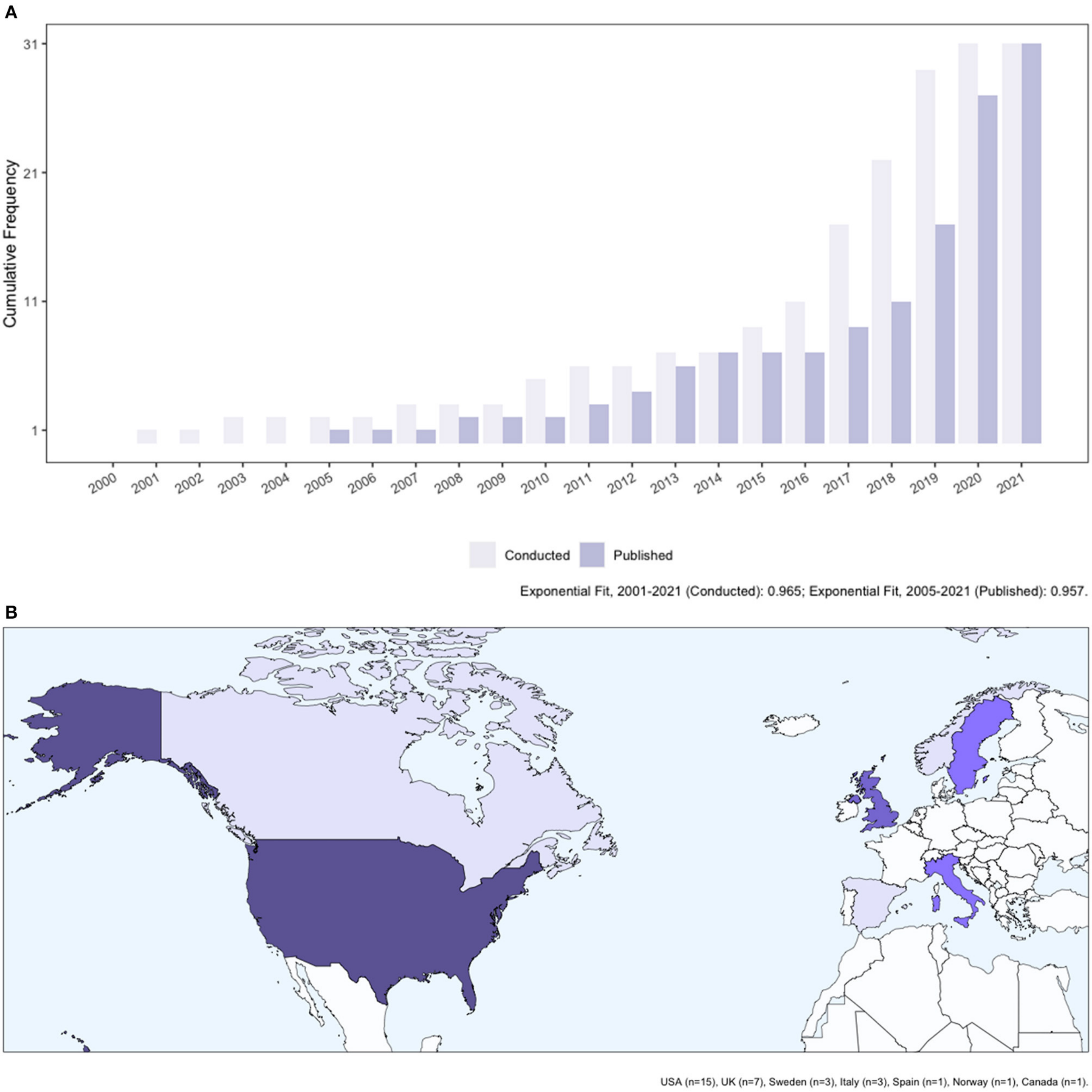


FIGURE 3
**(A)** Grouped bar graph plotting the annual increases in the number of meat reduction interventions implemented within university settings between 2000 and 2021. **(B)** Choropleth map illustrating the countries where, and frequency with which, included interventions were conducted between 2000 and 2021.

Italy (9.1%), Sweden (9.1%), Canada (3.0%), Spain (3.0%), Norway (3.0%), Scotland (3.0%), and Wales (3.0%).

## 3.2.2. Strategies and approaches

In total 51.6% of interventions targeted conscious decision-making processes ($n = 16$) while the remaining 48.4% targeted either the choice architecture of the retail environment ($n = 5$) or both drivers simultaneously ($n = 10$). Of the five strategies identified in our systematic review, promotional messaging was the most prominently used (80.6%), followed by strategies that modified the presentation and arrangement of items on menus (35.5%), manipulated the layouts of dining areas (9.7%), introduced pricing incentives (9.7%), and added to the existing set of meal options (8.3%).

In total, 61.3% of interventions utilized a single strategy in isolation while the remaining 12 used at least two in combination (38.7%). Among this latter group, promotional messaging was the most commonly integrated strategy (91.7%), followed by strategies that modified the presentation and arrangement of items on menus

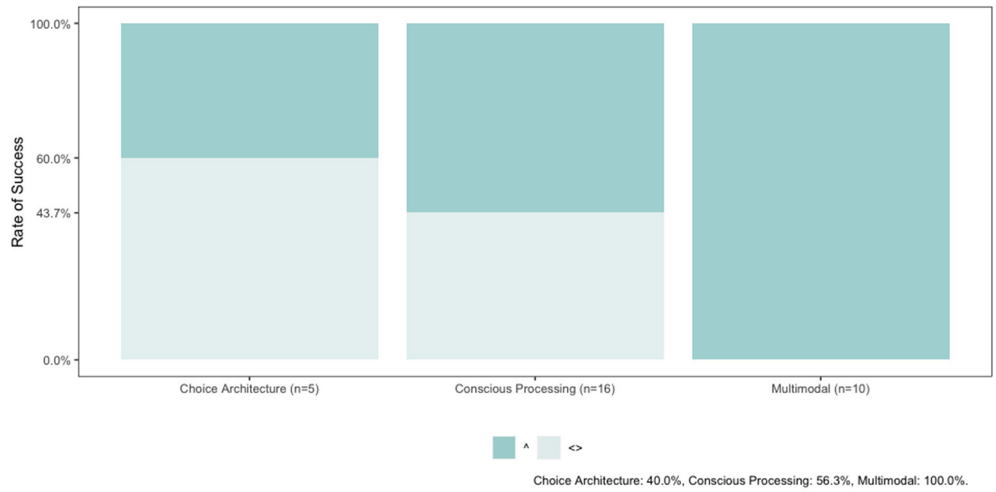


FIGURE 4
Grouped bar graph comparing the proportion of interventions associated with significant reductions in meat consumption across each investigated approach. Relative to other approaches, multimodal interventions were more likely to lead to significant reductions in the amount of meat consumed within university settings ($p = 0.029$). No increases in meat consumption were reported.

(83.3%), introduced pricing incentives (16.7%), manipulated the layouts of dining areas (8.3%), and added to the existing set of meal options (8.3%).

Interventions that manipulated the layouts of dining areas were the least likely to be implemented alongside at least one other strategy (33.3%). By contrast, interventions that utilized promotional messaging (44.0%), modified the presentation and arrangement of items on menus (90.9%), introduced pricing incentives (66.7%), and added to the existing set of meal options (50.0%) were all more likely to be integrated alongside another strategy.

### 3.2.3. Outcome variables and evaluation methods

Among the included interventions, differences in meat consumption were most often measured observationally (54.8%), with self-reported measures (32.3%) and measures of intention (12.9%) being used at a lower relative frequency. In tracking these changes, nine studies used between-group comparisons when estimating the effects of the intervention on food choice while the remaining 22 either evaluated within-group differences over time (22.6%) or used both evaluation methods concurrently (48.4%).

Over two-thirds of interventions targeted reductions in all types of meat (67.7%) while four focused on reductions in red and process meats (12.9%), three focused on reductions in ruminant meats (9.7%), two focused on red meat alone (6.5%), and one focused on processed meat alone (3.2%).

## 3.3. Quality assessment

All of the included studies satisfied at least one of the eight criteria specified by the Risk of Bias Tool, meaning that each study met the minimum standard of rigor for our main analyses (see Table 2). The mean summary score across all included studies was 3.19 ($sd = 2.17$), indicating a high degree of study-level variation. Among the evaluated criteria, 90.3% used a control or comparison group, 51.6% collected pre- and post-intervention data, 38.7% assessed equivalence between groups at baseline, 32.3% assessed equivalence across potentially relevant sociodemographic characteristics, 29.0% had a follow-up rate of at least 80%, and 25.8% randomly assigned participants for assessment. No study randomly selected participants for assessment.

The least applicable items were those assessing attrition (51.6%) and equivalence across potentially relevant sociodemographic factors (3.2%). The items that were most relevant to our studies, on the other hand, were those assessing equivalence across potentially relevant sociodemographic factors (61.3%) and equivalence between groups at baseline on disclosure (58.1%).

## 3.4. Main analyses

### 3.4.1. Success rate variations

Over two-thirds of the included interventions were associated with significant reductions in meat consumption (67.7%). The remaining interventions yielded no differences in behavior (32.3%), with none of the included studies reporting any increases in meat consumption resulting from negative reactance or rebound effects.

Between the three investigated approaches, multimodal interventions were significantly more likely to be associated with reductions in meat consumption than those targeting conscious decision-making processes or the choice architecture of the retail environment alone ($p = 0.029$) (see Figure 4). There was no

TABLE 2 Table summarizing the results of our quality assessment and risk of bias analysis.

| Study ($n = 31$) | Cohort | Control or comparison group | Pre/post intervention data | Random assignment of participants to the intervention | Random selection of participants for assessment | Follow-up rate of 80% or more | Comparison groups equivalent on sociodemographics | Comparison groups equivalent at baseline on disclosure |
|---|---|---|---|---|---|---|---|---|
| Andersson and Nelander (2021) | N | Y | N | N | N | NA | NR | NR |
| Brunner et al. (2018) | Y | Y | Y | N | N | NA | Y | Y |
| Campbell-Arvai (2011) | N | Y | N | Y | N | NA | NR | NR |
| Carfora et al. (2019) | Y | Y | Y | Y | N | Y | NR | Y |
| Carfora et al. (2016) | Y | Y | Y | Y | N | Y | Y | Y |
| Carfora et al. (2017) | Y | Y | Y | Y | N | Y | NR | Y |
| Cerezo-Prieto and Frutos-Esteban (2021) | Y | Y | N | N | N | NA | Y | NR |
| Dissen and Crowell (2020) | Y | Y | Y | N | N | Y | Y | Y |
| Garnett et al. (2021) | N | Y | N | N | N | NA | NR | NR |
| Garnett et al. (2020) | N | Y | N | N | N | NA | NR | NR |
| Garnett et al. (2020) | N | Y | N | N | N | NA | NR | NR |
| Garnett et al. (2019) | N | Y | N | N | N | NA | NR | NR |
| Hormes et al. (2013) | Y | Y | N | N | N | NA | NR | NR |
| Jalil et al. (2020) | Y | Y | Y | N | N | Y | Y | Y |
| Jay et al. (2019) | Y | Y | Y | N | N | Y | NR | Y |
| Kurz (2018) | N | Y | N | N | N | NA | NR | NR |
| Larner et al. (2021) | N | Y | N | N | N | NA | NR | NR |
| Malan (2020) | N | Y | N | N | N | NA | NR | NR |
| Malan et al. (2020) | Y | Y | Y | N | N | N | Y | N |
| McClain et al. (2013) | N | Y | N | N | N | N | NR | NR |
| McDonough (2012) | Y | N | Y | N | N | N | NR | NR |
| Michels et al. (2008) | N | N | Y | N | N | N | NR | NR |
| Nash (2014) | Y | Y | Y | Y | N | Y | N | Y |
| Papadaki and Scott (2005) | Y | Y | Y | N | N | N | Y | Y |
| Piester et al. (2020) | N | Y | N | Y | N | NA | Y | NR |

*(Continued)*





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TABLE 2 (Continued)

| Study ($n = 31$) | Cohort | Control or comparison group | Pre/post intervention data | Random assignment of participants to the intervention | Random selection of participants for assessment | Follow-up rate of 80% or more | Comparison groups equivalent on sociodemographics | Comparison groups equivalent at baseline on disclosure |
|---|---|---|---|---|---|---|---|---|
| Piester et al. (2020) | N | Y | N | Y | N | NA | Y | NR |
| Ring et al. (2019) | Y | Y | Y | N | N | Y | NR | Y |
| Schwitzgebel et al. (2020) | Y | Y | Y | N | N | Y | NR | Y |
| Slapo and Karevold (2019) | N | N | Y | N | N | NA | NA | NA |
| Turnwald and Crum (2019) | N | Y | N | N | N | NA | NR | NR |
| Wolstenholme et al. (2020) | Y | Y | Y | Y | N | N | Y | Y |

difference in the rate of success across interventions targeting the choice architecture of the retail environment and conscious decision-making process.

Interventions using at least two strategies concurrently were also more likely to be associated with reductions in meat consumption than interventions using a single strategy in isolation ($p = 0.024$), though both sets of interventions significantly reduced the amount of meat consumed within university settings on at least half of the evaluated occasions. Interventions that used promotional messaging strategies, in particular, were successful 57.1% of the time when used in isolation and 76.0% of the time when used in combination with other strategies ($p = 0.029$).

When comparing the performance between multimodal interventions and unimodal interventions leveraging two or more strategies, multimodal interventions were associated with a higher rate of success (100%, compared to 50.0%) and a greater overall effect on food choice ($OR = 2.88$ [1.95, 4.64]), compared to ($OR = 2.13$ [1.64, 3.05]).

There were no significant differences in the success rates associated with interventions conducted in Europe and North America ($p = 0.28$).

### 3.4.2. Effect size variations

The effect estimates associated with the included studies ranged from 17.97 [9.60, 33.63] to 0.61 [0.23, 1.58], with a mean standardized effect of 2.88 [1.85, 4.77], indicating that the included interventions reduced the overall odds of consuming meat within these settings by an average of 187.5%.

However, due to the heterogeneity in the mean effect estimates associated with studies using self-reported ($OR = 4.20$ [2.99, 5.88]) and intended ($OR = 4.14$ [2.23, 7.73]) measures of change, relative to those using observational measures of change ($OR = 1.82$ [1.37, 2.75]) (see Figure 5), we elected to use a fixed-effect model for our meta-analysis, limiting our comparisons to the 17 studies ($n = 17$) that used observational methods to evaluate changes in meat consumption (see Figure 6).

From this analysis, we found that interventions targeting the choice architecture of the retail environment had a greater mean effect on meat consumption ($OR = 1.82$ [1.57, 2.24]) than interventions targeting people's conscious decision-making processes ($OR = 1.68$ [1.43, 2.05]), though both approaches had a smaller average effect on meat consumption than multimodal interventions ($OR = 1.89$ [1.21, 3.41]) (see Figure 7). Across all approaches, this narrower set of interventions reduced the overall odds of consuming meat within university settings by 81.8%.

### 3.4.3. Time series variations

No significant differences were found in intervention performance over time.

## 4. Discussion

Prior research has indicated that organizational change can play a unique role in advancing sustainable development (Krabbe et al., 2015; Hamam et al., 2021; Garnett and Balmford, 2022; Nielsen,

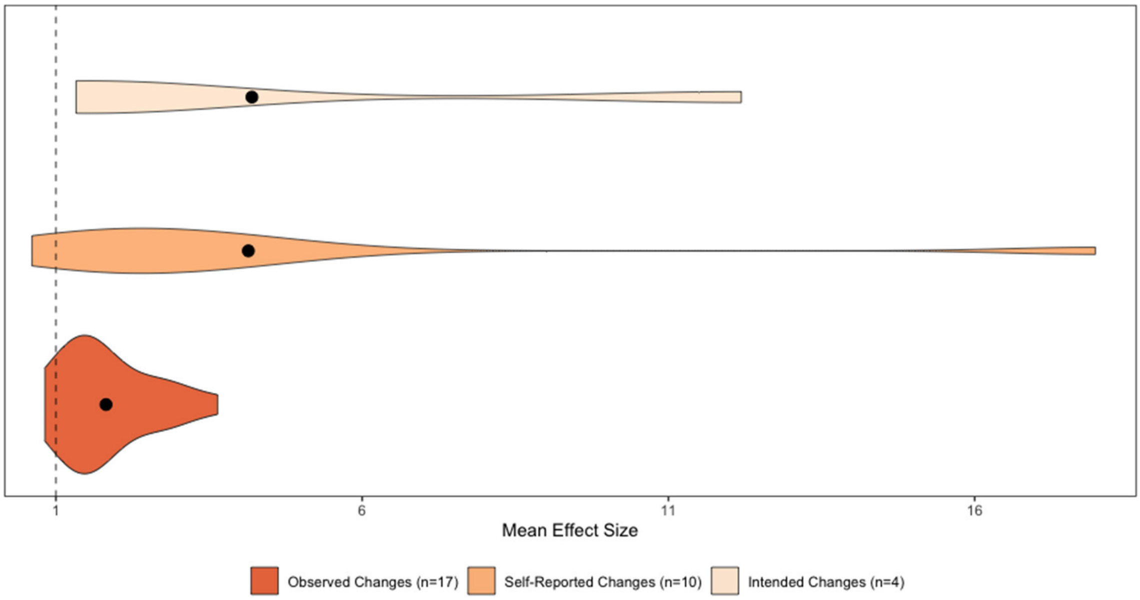


FIGURE 5
Violin plot comparing the effect size distributions for each of the three included evaluation methods. The differences in the magnitude of these effect estimates suggest some level of effect size heterogeneity across the three included methods of evaluation, with observational methods yielding smaller and more precise measurements of dietary behavior.

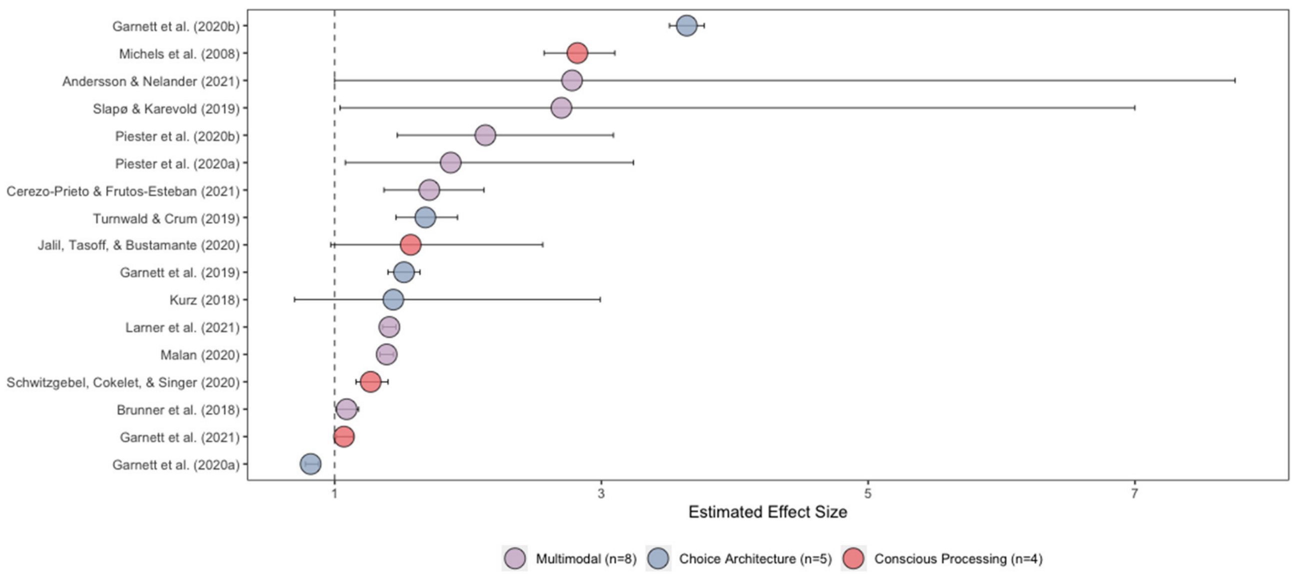


FIGURE 6
Forest plot comparing the effect estimates of the 17 studies included in the fixed effect model. Effects sizes are given as odds ratios, with error bars denoting 95% confidence intervals.

2023). In particular, there is an interest in understanding how HEIs can contribute to this larger effort by reducing the amount of meat consumed within campus environments (Ruiz-Mallén and Heras, 2020; Sherry and Tivona, 2022; Taylor et al., 2023). Despite the empirical progress that has been made over the last two decades, there has been little research to date reflecting on how useful these interventions have been in promoting voluntary changes in food choice within university settings. To address this gap in the literature, we synthesized the evidence on university-implemented meat reduction interventions to determine whether these interventions have been generally effective in promoting behavior change, and identify whether there are any relevant approach-dependent differences in intervention performance within these dining contexts. A summary of our findings and their implications

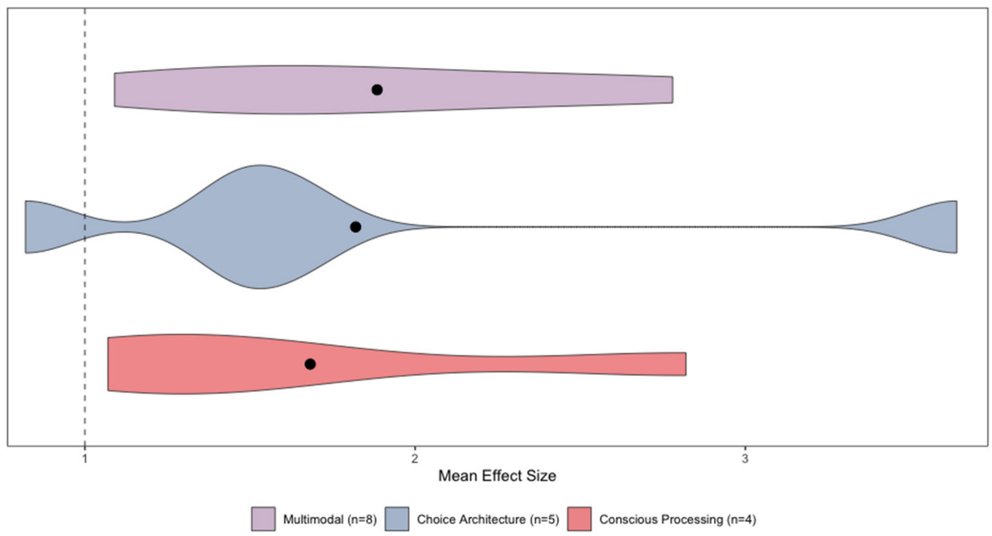


FIGURE 7
Violin plot comparing the effect size distributions of the three investigated approaches. Compared to the other two approaches, multimodal interventions had a greater overall effect on meat consumption.

for intervention design and dietary change research are given below.

## 4.1. Summary of main findings

In this systematic review of the university meat reduction literature, we identified and analyzed 31 dietary change interventions that had been implemented over the course of 21 years across 24 universities, nine countries, and two continents (see Figure 3). Of these interventions, over two-thirds led to significant changes in food choice (see Figure 4), lowering the overall odds of consuming meat within college and university settings by an average of 82% (see Figure 6).

Between the three approaches investigated in this systematic review (see Figure 1), multimodal interventions were found to be more reliable and effective in reducing meat consumption than interventions targeting the choice architecture of the retail environment or conscious decision-making processes alone (see Figure 7). This remained true even after controlling for the the number of individual strategies being used, indicating that these performance-related advantages were a function of more than just strategic volume. As such, from an intervention design standpoint, there may be inherent value in understanding how strategies can be integrated to exert influence on both implicit and deliberate decision-making processes.

The remaining two unimodal approaches were equally successful in reducing the amount of meat consumed within university settings (see Figure 3), though interventions exclusively targeting the choice architecture of the retail environment were found to have a greater mean effect on food choice than interventions targeting conscious decision-making alone (see Figure 7).

Over the evaluated 21-year period, there was an exponential increase in the amount of research that was conducted and published on university-implemented meat reduction interventions (see Figure 3). Despite this proliferation, time series analysis revealed no salient improvements in intervention performance over time, highlighting a possible need for more effective, setting-specific guidelines for reducing meat consumption within university settings.

Our findings on the overall success of university-implemented meat reduction interventions were largely consistent with prior research examining the value of behavior change strategies in promoting reductions in meat consumption (Bianchi et al., 2018a,b; Harguess et al., 2020; Kwasny et al., 2022; Ronto et al., 2022). However, the comparatively higher rate of success observed in this study relative to previously published reviews could be attributed to the differences in the populations being targeted by included interventions, with earlier reviews investigating the broader effects of behavior change strategies on the general population and ours focusing instead on changes in food choice among mostly young adults and adolescents, who have been found to be more receptive to plant-rich diets and dietary change interventions, more broadly (de Villiers and Faber, 2019; Hargreaves et al., 2021; Jürkenbeck et al., 2021).

Our comparisons of the three approaches investigated in this study provide an intuitive, decision-centered framework for implementing meat reduction interventions within university settings. More specifically, our study contrasts earlier work on the subject by using dual-process accounts of conditional reasoning to typify interventions based on the cognitive processes targeted by their strategies (Evans, 2011; Kahneman, 2011), rather than the various sources of influence involved in dietary decision making (i.e., intrapersonal-, interpersonal-, organizational-, community-, and policy-level factors). While socioecological perspectives provide a useful method of conceptualizing how different contextual factors interact to influence food choice at a systems level (Robinson, 2008; Townsend and Foster, 2013), they tend to be less instructive for intervention design when the desired changes in behavior (i.e., reductions in meat consumption) are voluntary and the dining context (i.e., university cafeterias) is fixed (Schölmerich and Kawachi, 2016). The classification scheme used in this study circumvents these limitations by focusing instead on how common meat reduction strategies differentially inform decision-making processes at the individual level.

The results from our joint analysis were mostly consistent with earlier research supporting the value of integrated approaches to meat reduction (Kahn-Marshall and Gallant, 2012; Gittelsohn and Lee, 2013; Vo et al., 2019; Ramsing et al., 2021). In addition to replicating these general findings, we were also able to use our novel classification scheme to distinguish between three different types of meat reduction approaches, with multimodal interventions outperforming interventions targeting the choice architecture of the retail environment and conscious decision-making processes alone, independent of the number of strategies involved. While past research has suggested that the advantages to integrated approaches are a result of probability (Kahn-Marshall and Gallant, 2012) and focused efforts to leverage links between socioecological levels (Schölmerich and Kawachi, 2016), our findings seem to suggest an alternative explanation—that the benefits to performance are a function of both the number of strategies used and the nature of how those strategies coalesce to exert influence on relevant decision-making processes.

With respect to the remaining two approach categories, our findings on interventions targeting the choice architecture of the retail environment were consistent with prior research supporting nudging interventions as an effective way of promoting dietary change (Bucher et al., 2016; Byerly et al., 2018; Vandenbroele et al., 2020; Ensaff, 2021; Mertens et al., 2022). However, for interventions targeting conscious decision-making processes, the research has been more mixed (Worsley, 2002; Bianchi et al., 2018a; Thakur and Mathur, 2021), with some doubts being raised about the sufficiency of education-based strategies in influencing actual behavior within applied contexts (Kaur et al., 2017). Despite the salience of these concerns, we did not find evidence of this in our analyses, (see Figure 4), with interventions targeting conscious decision-making processes yielding significant changes in intention as well as behavior in 56% of cases. However, we did find that interventions targeting conscious decision-making processes did tend to perform better when they were combined with at least one other strategy.

Finally, the exponential increase reported in the number of university-implemented meat reduction interventions mirrors the increase in meat reduction interventions that have been implemented more generally over the last several decades (Kwasny et al., 2022). It is therefore unclear how much of this can be attributed to the establishment of institutional network programs, like the Association for the Advancement of Sustainability in Higher Education (AASHE) and the United Nation's Higher Education Sustainability Initiative (HESI), and how much can be attributed to increasing public concern and awareness surrounding the environmental, climate, and health implications of food (Macidarmid et al., 2016; Jürkenbeck et al., 2021). Despite the increasing awareness of the interactions between agriculture, diet, public health, and the global ecology, we found no evidence pointing to any increases in the relative performance of university-implemented meat reduction interventions over time.

## 4.2. Strengths and limitations

To the best of our knowledge, our study is the first to critically examine the value of university-implemented meat reduction interventions, and the first to jointly analyze the relative merits between these three approaches to dietary change. We accomplished this using a novel, theory-informed classification scheme that typifies interventions according to the decision-making processes targeted by their individual strategies, allowing us to make nuanced comparisons between interventions that consciously incentivize individuals to make more sustainable food choices, interventions that manipulate the physical environment in ways that make those choices easier, and interventions that do both simultaneously. In doing so, we were able to identify which approach was most effective in promoting dietary change within these settings while also providing supporting, evidence-based explanations highlighting the behavioral mechanisms that could be involved in driving these observed effects. In addition, our evaluations of these meat reduction approaches made use of two distinct performance-related outcomes: one focused on the capacity of interventions to generate significant reductions in meat consumption and one focused on measuring the degree to which change occurred within these university environments. By distinguishing between these outcomes, we were able to leverage the existing evidence to compare the performance of these interventions across multiple dimensions. Finally, by undertaking this exercise, we were also demonstrate how settings-based evaluation techniques can be used to meaningfully inform the design of setting-specific interventions and policies.

However, within the context of this study, we were unable to evaluate the long-term effects of university meat reduction efforts on food choice. Due to the limited duration of the included studies' evaluation periods, we were unable to assess the how long these changes in behavior persisted within these environments, and whether their persistence was at all contingent on the type of approach used. This limitation is especially salient given the existing empirical concerns surrounding the durability of nudging individuals toward healthier food options (Van Rookhuijzen et al., 2021). For the same reasons, we were also unable to evaluate whether these interventions were associated with any rebound effects resulting from psychological reactance (Osman, 2020). Furthermore, because the included studies evaluated behaviors that were specific to university environments, it is also unclear whether the benefits of implementing these types of interventions within university environment led to meaningful instances of contextual spillover (Verfuerth et al., 2021), or if they induced change across other desirable pro-environmental behaviors (Carrico, 2021). Finally, while we identified meat reduction interventions that had been implemented in universities across nine different countries, all of these countries are situated in either Europe or North America. Therefore, it remains unclear whether these findings are generalizable across other cultural contexts.

### 4.3. Recommendations for future research

To better account for the asymmetries in the effect sizes associated with each of the three included outcome measures, future investigations should prioritize using observational methods to track changes in dietary behaviors where possible. In addition to providing more precise approximations of changes in food choice (Webb and Sheeran, 2006; Loy et al., 2016; Meyer and Simons, 2021) and minimizing the risks of bias that stem from the so-called "intention-behavior gap," collecting observational data has the additional benefit of equipping institutional policymakers and foodservice providers with a practical means of making complementary supply-side changes based on information that is collected at the point of purchase. Furthermore, to allow for a better sense of whether the investigated approaches can lead to lasting changes in behavior, researchers should endeavor to evaluate the effects of these initiatives more frequently and over longer intervals of time. In addition to allowing investigations to be more sensitive to instances of reactance, higher monitoring frequency may also allow researchers to pick up on uninvestigated patterns in meat consumption, like those resulting from seasonality, that may inform how these types of meat reduction interventions could be more strategically timed. Furthermore, researchers could additionally use survey items to understand whether these approaches are associated with meaningful contextual spillover effects, or if they lead individuals to engage in other pro-environmental behaviors unrelated to meat consumption. Finally, the question of how generalizable these effects are across other cultural contexts and institutional settings remains unanswered. Future research should therefore investigate whether settings-based evaluation techniques may prove useful across similar dining contexts (Moore et al., 2013; Hertwig and Grüne-Yanoff, 2017), such as in hospital and workplace cafeterias, and whether these findings can be replicated in universities that stand to benefit from these interventions that are situated outside of a Western context, such as in China and Brazil.

## 5. Conclusions

The results from this systematic and meta-analysis provide compelling evidence in favor of university-implemented meat reduction interventions and their value in promoting dietary change within university settings. Through our comparisons of the different approaches that have been used within these environments, we were able to identify a number of strategic advantages associated with using multimodal interventions to facilitate these desired changes in food choice. Institutional stakeholders interested in engaging in these types of sustainable dining initiatives should therefore consider incorporating these design principles into future interventions. Despite the promise of these initial findings, further research is still needed to understand how long these effects endure within university environments, and whether these design principles are generalizable across other settings and cultural contexts.

## Data availability statement

The datasets presented in this study can be found in the following online repositories: [link 1] and https://github.com/kenjinc/university-meat-reduction-srma/blob/main/markdowns/visuals.md.

## Author contributions

KC initiated and conceptualized the project. LR designed and implemented the search strategy with assistance from KC and AW. KC and AW conducted the relevant screening procedures with assistance from DA-J and RR. KC synthesized the extracted data, conducted the relevant analyses, and drafted the manuscript. RR acquired funding for the project and provided administrative support and supervision throughout. All authors reviewed the final version of the manuscript prior to submission.

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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.2023.1103060/full#supplementary-material

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# Phytochemical composition and antioxidant activities of some wild edible plants locally consumed by rural communities in northern Uganda

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**Background:** *Acalypha rhomboidea*, *Asystacia gangetica*, *Crassocephalum sacrobasis*, *Crotalaria ochroleuca*, *Heterosis rotundifolia*, *Hibiscus cannabinus*, *Hibiscus* sp., *Hibiscus surratensis*, *Ipomoea eriocarpa*, *Maerua angolensis*, *Senna obtusifolia* and *Vigna membranacea* are among the common wild edible plants in the Acholi sub-region, northern Uganda. This study evaluated the phytochemical constituents and antioxidant potential of the plants.

**Methods:** Fresh leaves collected from each plant species were air-dried under shade. The phytochemical contents of the ethanol and petroleum ether extracts were determined using standard protocols. The antioxidant content of the methanolic extracts was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

**Results:** Preliminary phytochemical analyses indicated the presence of tannins, reducing compounds, alkaloids, flavonoids, flavons aglycones, flavanosides, anthracenosides, anthocyanosides, volatile oils, coumarins, steroid glycosides, sterols and triterpenes. However, the extracts did not contain any emodols and saponins. The results of the quantitative phytochemical analysis showed that the contents of different phytochemicals detected varied significantly ($p<0.05$) among the selected plants. The amount of tannins in mg/g (gallic acid equivalent) of dry weight varied from 3.90±0.16 in *C. ochroleuca* to 10.41±0.78 in *I. eriocarpa*, total flavonoids in RE, mg/g dry matter from 4.07±0.11 in *I. eriocarpa* to 14.94±0.08 in *S. obtusifolia.* Total alkaloids in mg/100g ranged from 1.59±0.30 in *I. eriocarpa* to 6.37±0.24 in *Hibiscus* sp. Total phenolic content in GAE, mg/g dry matter ranged from 13.39±0.26 in *A. rhomboidea* to 64.25±0.54 in *I. eriocarpa*. The *in vitro* antioxidant assays revealed substantial free radical scavenging activity in all the plants. Antioxidant activity expressed as $IC_{50}$ (ppm) ranged from 13.39 for *A. rhomboidea* to 64.84 for *I. eriocarpa,* compared to 12.82 for ascorbic acid standard. The total phenolic compounds and total tannins had significant and positive correlations with DPPH free radical scavenging activity.

**Conclusion:** The findings of this study provide evidence that the species are good natural sources of phytochemicals and antioxidants, whose regular consumption could provide human health benefits by protecting against oxidative stress related diseases. Further research is needed on the structural characterization of the phytochemicals, profiling the plant extracts with high antioxidant activity and determining the antimicrobial activities.

KEYWORDS

polyphenolic compounds, phenolics, methanolic extracts, *in vitro* antioxidant activity, wild leafy vegetables, radical scavenging activity

## Introduction

Many countries worldwide, including those in sub-Saharan Africa, are experiencing increased incidences of chronic non-communicable diseases. This is partly due to unhealthy diets (1, 2). It has been established that consumption of wild edible plants can lower the incidences of some non communicable diseases (3), because they contain a variety of phytochemicals that have therapeutic potentials and can prevent many human diseases including cancer and cardiovascular diseases occurring (4, 5).

Phytochemicals are secondary plant metabolites that include phenolic compounds such as tannins, flavonoids, saponins and glycosides (6–9). Although they have no known roles in plant cell metabolism, the plants continuously synthesize phytochemicals for defense roles and to protect plants from possible environmental harm, e.g., against pathogens and herbivores attacks (10, 11). Many phytochemicals have proven human health benefits (12). For instance, phenolic compounds are of great importance due to their potent antioxidant or free radical scavenging activities (13, 14), antiseptic properties (15) and anti-inflammatory roles (16, 17). Alkaloids are powerful drugs with anti-inflammatory, antimalarial, antimicrobial and antispasmodic activities. Similarly, phytosteroids are known to have cardiotonic, antibacterial (18) and mixed agonistic/antagonistic activity to animal steroid receptors (19). Anthocyanins are flavonoid compounds that help the immune system to work more efficiently to protect against viral infections (20, 21). The phenolic compounds display antioxidant activities, which are important for healthy functions in the cells of both plants (22), and humans (23, 24).

Antioxidants are free radical scavengers that act by donating electrons to the electron-deficient free radicals, thus, rendering the radicals harmless (25–27). These free radicals include reactive oxygen species (ROS) such as superoxide anion radical and hydroxyl radical, hydrogen peroxide and singlet oxygen formed from metabolism (28) and reactive nitrogen species such as nitric oxide and peroxynitrite. Metabolic reaction in the cell mitochondria is the body's main source of free radicals (29, 30). Exogenous sources of free radicals include exposure to chemical contaminants in food, charred food, cosmic radiation from space and smoking (31). A myriad of metabolic reactions in the body involves synthesizing various chemical intermediates susceptible to free radical attacks (32, 33). Free radicals' interference in the body reactions alters amino acid configurations, denaturing enzymes. Free radicals attack nucleic acids, breaking down the nucleotide strands when they occur at the sugar linkages. Free radicals can also play helpful roles in some instances, such as apoptosis (34). For healthy body functions, there must be a balance between generating and removing free radicals. Many oxidative stress-related diseases, such as heart diseases and cancer (35, 36) result from the accumulation of free radicals in the body. Therefore, removing or scavenging free radicals significantly reduces the oxidative stress or imbalance between the free radicals generation and their removal in the body. Moderate amounts of ROS play an essential role in cell signaling involving apoptosis and gene expression and can serve as both intra- and inter-cellular messengers (37–39).

Several wild edible plant species are utilized by the Acholi communities in northern Uganda (40). Despite the abundance and diversity of these plants, many of them have not been explored for their phytochemical and antioxidant properties (41). The phytochemical and antioxidant screening of these plants is a important for verification of their continued consumption and future utilization as health-promoting foods. Therefore, this study aimed to analyse; (i) the phytochemical constituents, (ii) *in vitro* antioxidant potential of extracts of selected wild edible plant species consumed by the Acholi communities in northern Uganda, and (iii) to correlate the phytochemical contents with the antioxidant activities.

## Materials and methods

### Collection of plant materials

Fresh leaves (about 1 kg) of each selected wild edible plants, named as *Acalypha rhomboidea* Raf., *Asystacia gangetica* (L.) T. Anderson, *Crassocephalum sacrobasis* (DC.) S. Moore, *Crotalaria ochroleuca* G. Don., *Heterotis rotundifolia* (Sm.) Triana, *Hibiscus cannabinus* L., *Hibiscus* sp., *Hibiscus surratensis* L., *Ipomoea eriocarpa* R.Br., *Maerua angolensis* DC., *Senna obtusifolia* (L.) Irwin & Barneby and *Vigna membranacea* A. Rich., were collected at the flowering stage following the standard guidelines (42) for plant sample collection from locations in Omoro district in northern Uganda (Figure 1) between November 2019 and March 2020. Based on a previous record of Ugandan wild edible plants by Goode (43), these plants are used as a vegetable to accompany staple foods during periods of food scarcity. *Acalypha rhomboidea* is an annual garden weed used during food scarcity around April–May. *Asystica gangetica* (Ladyelcol), family Acanthaceae is a native wild-leafy vegetable that mostly grow in forest habitats. It grows up to 1 m high with extensive branching during the wet season. *Crassocephalum sacrobasis* is an annual weed of the garden used around April–May. *Crotolaria ochrolenca* (locally called Lawija) is an erect much-branched or short-lived leaf biennial vegetable herb growing up to 2.5 m tall. *Heterosis rotundifolia* (locally called Odwanga/Cunbit) is a herbaceous flowering plant found in wetlands and riverbanks. *Hibiscus cannabinus* (Lagoroto in Acholi) from the family Malvaceae is an annual herbaceous dicotyledonous wild plant. The shoots or young leaves and sometimes the flowers and young fruits are used as vegetables during the wet season. *Hibiscus surratensis* (Gwanya in Acholi), belongs to the family Malvaceae and grows in bushes around wetlands. *Hibiscus* sp. (Nyarogenga in Acholi) is an annual herb in the Malvaceae family which grows up to 1.5 m high. *Ipomoea*

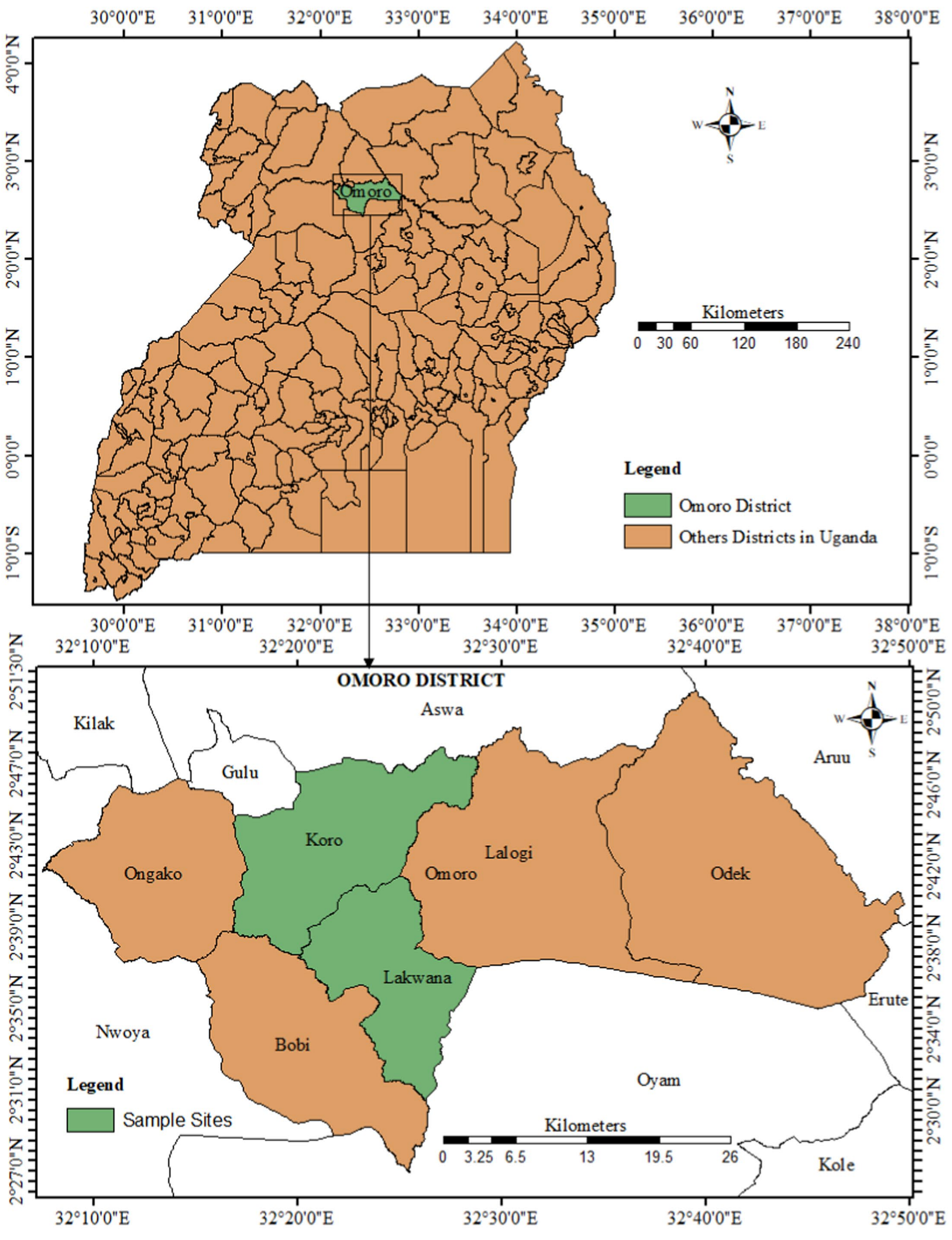


FIGURE 1
Location of study areas in Omoro district, northern Uganda. The map was created by the authors using ArcGIS version 10.3.1.

*eriocarpa* (Padowiakuri), from the family Convolulaceae, is an annual crawling herb traditionally used as a leaf vegetable in Uganda. *Maerua angolensis* (locally called Odwee) is a shrub or small tree usually growing up to 4 m high in savannah and galleried forest areas of tropical Africa. The leaves and tender parts are eaten as a vegetable in times of food shortage during the dry season. *Senna obtusifolia*, locally called Oyado in Acholi. It is an erect and bushy biennial or short-lived perennial herb in the family Fabaceae growing to a height of 1.5–2.5 m. The leafy vegetable is an important source of food for rural populations during the wet season. *Vigna membranacea* (locally called Boo Ayom) is a climbing cow-pea like plant/vine common on cultivated land and bushland. The leaves are used as vegetable during the rainy season, March–May and November–January. The plant samples were transported in clean polythene bags to the laboratory. Taxonomic identification of these plant species was made by a plant taxonomist Mr. Rwambindore Protease at the Makerere University Herbarium in a previous study Nyero et al. (40).

## Sample preparation and extraction

The leaves were removed and gently washed to remove any debris, air-dried under shade at room temperature for 5 to 10 days in the laboratory. The dried plant samples were ground to a fine powder using an electric grinder, sieved and packed in a polyethylene plastic bag wrapped with aluminum foil. One hundred grams of powder from each plant material plant samples were extracted using 500 ml of general-purpose grade petroleum ether (40°C) in an extraction flask with periodic stirring for 3 days at room temperature (20–25°C). The mixture was filtered over Whatman No.1 paper using Büchner funnel. The residue was dried in the fume hood until the smell of petroleum ether was removed. The dried residue was soaked in 70% ethanol, stirred for 30 min and left for 24 h with periodic shaking (44, 45). Then the extracts were filtered using Whatman No. 1 filter paper and the filtrate evaporated to dryness under vacuum using a rotary evaporator at 60°C. The crude extract was stored in a refrigerator below 4°C for subsequent analysis.

## Phytochemical screening of the plant extracts

Phytochemical screening of the crude petroleum ether and ethanol extracts of all plants for the presence or absence of tannins, saponins, reducing compounds, alkaloids, flavonoids, flavons aglycones, flavanosides, anthracenosides, anthocyanosides, volatile oils, courmarins, emodols, steroid glycosides, sterols and triterpenes was carried out using standard methods (7, 46–49) with minor modifications.

# Quantification of phytochemicals

## Determination of total tannin contents

Total tannins were determined using the Folin–Ciocalteu method (50) with minor modifications. Plant powder (0.1 g), from each sample was extracted in 10 ml of distilled water. In each case, the extract (0.1 ml) was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water. A drop of Folin–Ciocalteu reagent (0.5 ml) and 35% sodium carbonate (1 ml) solution were added. The mixture was diluted to 10 ml with distilled water, shaken and kept in the dark at room temperature for 30 min. Absorbances for the test and standard solutions were measured against distilled water as a blank at 725 nm. The regression equation $Y = 0.39X + 0.023$; $R^2 = 0.999$ obtained from the gallic acid standard curve (0, 10, 20, 40, and 50) μg/ml was used to express the results as mg equivalents of gallic acid, GAE/g.

$$C = C_1 \frac{v}{m}$$

C = total tannin in terms of gallic acid equivalent (GAE) mg/g, $C_1$ = concentration of gallic acid derived from the standard curve, $v$ = volume of extract in milliliters, and $m$ = weight of plant extract in grams.

## Determination of total phenolic content (TPC)

The total phenolic content (TPC) in each extract was determined using the Folin–Ciocalteau method (51), with minor modifications. Gallic acid was used as a standard. The plant powder (0.1 g) was extracted in 10 ml of distilled water for each sample. Then 0.5 ml of Folin–Ciocalteau reagent was added to 0.1 ml of each plant extract solution. The total volume of the extract was reconstituted to 8.5 ml with distilled water, shaken and the resulting mixture was kept at room temperature in the dark for 10 min. Then 20% sodium carbonate (1.5 ml) solution was added, mixed thoroughly. The solution was allowed to stand in a water bath at 40°C for 20 min. Finally, the absorbance was measured at 755 nm using a UV-spectrophotometer. The gallic acid standard curve $Y = 0.014X + 0.036$; $R^2 = 0.997$ was obtained using dilutions (0, 10, 20, 40, and 50 μg/ml) for measuring the TPC, which was expressed as mg of gallic acid equivalent per gram (mg GAE/g) of dry sample. TPC was determined and expressed as mg of gallic acid equivalent per gram (mg GAE/g) of dry sample (52).

## Estimation of total flavonoid content (TFC)

The total flavonoid content (TFC) in each sample was determined using the Aluminium Chloride colorimetry method described by Ordonez et al. (53). Rutin was used as a standard and TFC was determined in milligrams of rutin equivalent (mg RE/g dry weight of extract). The calibration curve $Y = 0.10X + 0.018$; $R^2 = 0.991$ for rutin was obtained using different dilutions (0, 10, 20, 40, and 50 μg/ml) prepared in distilled water. Each plant powder (0.1 g) was extracted in 10 ml of 80% methanol. To 0.1 ml of the extract or standard, 0.5 ml of fresh aluminium chloride ($AlCl_3$, 2%) in ethanol was added. After 1 h at room temperature, the absorbance of the reaction mixture was measured at 420 nm using a UV–visible spectrophotometer. All results were recorded from triplicate samples.

## Alkaloids content determination

Total alkaloid content was evaluated gravimetrically using a standard method (54). Five grams of the powdered sample of each plant was weighed into a 200 ml of 10% acetic acid in ethanol and allowed to stand for 4 h. The filtered extract was concentrated using a water bath at 55°C to one-quarter of the original volume. Concentrated ammonium hydroxide ($NH_4OH$) was added dropwise into the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate collected was washed with dilute $NH_4OH$ solution and then filtered. The crude alkaloid residue was weighed and calculated according to the equation: Amount of alkaloid (mg/g) = weight of precipitate/weight of the sample (55).

## Antioxidant assay

The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with ascorbic acid standard according to the method described by Abdul-Wahab et al.

(56) with minor modifications. Each plant powder sample (0.1 g) was cold extracted with 10 ml of methanol (99%) for 3 h by soaking, shaking intermittently and later filtered. The volume of the extract solutions was adjusted to 10 ml with methanol to make sample solutions. The sample solutions (100, 200, 300, 400, and 500 μl) and 5 ml of 0.0039% DPPH were rapidly mixed in the test tubes. After vigorous shaking, the reaction mixture was incubated in the dark at 37°C for 30 min and the absorbance was measured at 517 nm against a blank using a UV–Vis. Spectrophotometer. The ascorbic acid standard curve ($Y = 1.468X + 31.17$; $R^2 = 0.993$) made from serial concentrations (5, 10, 15, and 20 μg/ml) was used as a positive control. The DPPH free radicals scavenging activity of the plant extract was expressed in terms of the effective concentration in parts per million of ascorbic acid standard and samples required to scavenge 50% of DPPH free radicals *in vitro*, $IC_{50}$; using the equation:

$$\text{DPPH free radical scavenging activity}\left(\%\right) = \left(\frac{A_c - A_s}{A_c}\right) \times 100$$

Where $A_c$ = Absorbance of the blank or control solution (i.e., the absorbance of DPPH + methanol); As is the absorbance of DPPH radical + sample (i.e., the absorbance of extract or standard). The $1C_{50}$ values were determined from the plotted graph of the percentage of scavenging activity against the concentration of different extracts from the three replicates. A lower $IC_{50}$ value implies a higher antioxidant activity of the sample.

### Statistical analyses

One-way ANOVA was performed to determine the significant differences between means. A *p* value < 0.05 was considered statistically significant. Then Tukey's HSD *post hoc* comparison test was done. Linear regression analysis between the % inhibition of DPPH and the concentration was done for each sample ($n = 3$) using a linear function. The Pearson's correlation coefficient was performed to assess the association of total phenolic, flavonoid, alkaloid and tannin contents and antioxidant activity. The statistical analysis was conducted using SPSS programme (IBM SPSS statistics version 26) for windows software. The results were presented as mean values ± SD (standard deviation).

## Results

### Phytochemical constituents

Preliminary phytochemical screening of petroleum ether and ethanol extracts from the 12 wild edible plants revealed the presence of a wide range of phytochemical compounds including volatile oils, basic alkaloids, and tannins, reducing compounds, coumarins, flavone aglycones, flavanoside, steroid glycoside, sterols and triterpenes, others occurring in higher abundance is some of the extracts as shown in Table 1. Anthracenosides were only present in two sample extracts: *H. surratensis* and *I. eriocarpa*. Anthocyanosides were found in the extracts of *H. surratensis*, *H. cannabinus*, *S. obtusifolia*, *Hibiscus* sp. and *I. eriocarpa*. None of the extracts contained saponins and emodol (Table 1).

Statistical analysis results of detected quantitative phytochemical contents of the 12 selected plants indicated that they were significantly different ($p < 0.001$; Table 2). As shown in Table 1, the total tannin content ranged from of 3.90 ± 0.16 in *C. ochroleuca* to of 10.41 ± 0.78 GAE, mg/g in *I. eriocarpa*. Total flavonoids varied from 4.07 ± 0.11 to 14.94 ± 0.08 RE, mg/g. The highest amount of flavonoids (14.94 ± 0.08 mg RE/g dry weight) was observed in extracts of *S. obtusifolia* while the lowest amount (4.07 ± 0.11) was detected in *I. eriocarpa*. The total phenolic content in all the plant species ranged from 13.39 ± 0.26 to 64.25 ± 0.54 mg GAE/g dry matter. *Ipomoea eriocarpa* exhibited the highest amount of TPC with 64.25 ± 0.54 GAE/g dry matter, meanwhile *A. rhomboidea* had the lowest (13.39 ± 0.26). Total alkaloids (g/100 g) varied from 1.59 ± 0.30 to *I. eriocarpa* to 6.37 ± 0.24 in *Hibiscus* sp. (Table 2).

### 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

Among the 12 plants investigated, *A. rhomboidea* exhibited the strongest free radical scavenging ability and may be used as a potential source of natural antioxidants against free radical-associated diseases. The DPPH radical scavenging of the plants determined as $IC_{50}$ (ppm) ranged from 13.39 to 64.84 compared to 12.82 for the standard ascorbic acid used (Table 2).

### Correlation of antioxidant activity with the phytochemical contents

The results of Pearson correlation revealed a significant and positive correlation of antioxidant or DPPH radical scavenging activity ($IC_{50}$) with total tannins ($r = 0.600$, $p = 0.039$) as well as total phenolics ($r = 0.999$, $p < 0.001$). However, there were no significant correlations with total flavonoids ($r = -0.238$, $p = 0.457$) and total alkaloid contents ($r = 0.052$, $p = 0.873$).

## Discussion

The results show that the leaves of wild edible plants consumed by the Acholi communities in northern Uganda contain a wide range of phytochemicals. All the 12 wild edible plants contained some tannins. The tannin content ranged from 3.9 to 10.4 mg/100 g. These values are higher than those in similar studies with comparable methods; for instance, Senguttuvan et al. (57) reported the tannins content of the dry powder of wild edible plants collected from Nilgiris, the Western Ghats, Tamil Nadu, India to range from 0.03 and 1.62 mg GAE/100 g. Emmanuel et al. (58) reported lower tannin content in wild edible plants from Iringa district, Tanzania, ranging from 1.05 to 19.02 mg/100 g. The observed differences could be explained by differences in geographical distribution and variability of soil nutrients (59, 60). Although tannins have traditionally been

TABLE 1 Phytochemical screening of crude extracts of 12 edible wild plant species used locally by the Acholi communities of northern Uganda.

| Sample | AR | HS | CO | VM | HC | SO | AG | H | HR | IE | CR | MA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volatile oils | + | + | + | + | + | + | + | + | + | + | + | + |
| Basic alkaloids | + | + | + | + | + | + | + | + | + | + | + | + |
| Sterols and triterpenes | + | + | + | + | + | + | + | + | + | + | + | + |
| Emodols | – | – | – | – | – | – | – | – | – | – | – | – |
| Coumarins | + | ++ | + | ++ | ++ | ++ | + | ++ | + | ++ | ++ | + |
| flavone aglycones | ++ | ++ | + | ++ | ++ | ++ | + | ++ | ++ | ++ | ++ | ++ |
| Saponins | – | – | – | – | – | – | – | – | – | – | – | – |
| Tannins | + | + | + | + | + | + | + | + | + | + | + | + |
| Reducing compounds | + | + | + | + | + | + | + | + | ++ | + | ++ | + |
| Alkaloid salts | + | + | + | + | + | + | + | + | + | + | + | + |
| Anthracenosides | – | + | – | – | – | – | – | – | – | + | – | – |
| Anthocyanosides | – | + | – | – | + | + | – | + | – | + | – | – |
| Flavonosides | ++ | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Steroid glycosides | + | + | + | + | + | + | + | + | + | + | + | + |

AR = *Acalypha rhomboidea* Raf., AG = *Asystasia gangetica* (L.) T. Anderson, CR = *Crassocephalum rubens* var. *sarcobasis (Bojer ex DC.)* C. Jeffrey & Beentje, CO = *Crotalaria ochroleuca G.Don.*, HR = *Heterotis rotundifolia (Sm.) Jacq.-Fél.*, HC = *Hibiscus cannabinus L.*, HS = *Hibiscus surattensis L.*, H = *Hibiscus* sp., IE = *Ipomoea eriocarpa R. Br.*, MA = *Maerua angolensis*, SO = *Senna obtusifolia* (L.) Irwin & Barneby., and VM = *Vigna membranacea A. Rich.*; (– absence, + presence, ++ present in large amount based on color intensity from the test outcome).

TABLE 2 Total tannins, total flavonoids, total phenolic compounds, alkaloids and antioxidant activity of methanol extracts of 12 selected wild edible plants from the Acholi sub-region.

| Sample | Total tannins (GAE, mg/g) | Total flavonoids (RE, mg/g) | Total phenolic compounds (GAE, mg/g) | Total alkaloids (g/100g) | Antioxidant activity ($IC_{50}$) |
|---|---|---|---|---|---|
| *Asystacia gangetica* | $3.95 \pm 0.06^{a}$ | $7.66 \pm 0.20^{d}$ | $14.18 \pm 0.63^{a}$ | $2.75 \pm 0.08^{bc}$ | 14.18 |
| *Acalypha rhomboidea* | $7.30 \pm 0.54^{b}$ | $5.17 \pm 0.07^{b}$ | $13.39 \pm 0.26^{a}$ | $1.95 \pm 0.22^{ab}$ | 13.39 |
| *Crassocephalum sacrobasis* | $7.50 \pm 0.33^{b}$ | $6.48 \pm 0.20^{c}$ | $32.54 \pm 1.33^{d}$ | $4.02 \pm 0.40^{d}$ | 32.54 |
| *Crotalaria ochroleuca* | $3.90 \pm 0.16^{a}$ | $11.00 \pm 0.09^{f}$ | $13.72 \pm 0.27^{a}$ | $4.92 \pm 0.29^{e}$ | 13.72 |
| *Heterotis rotundifolia* | $4.83 \pm 0.36^{d}$ | $6.69 \pm 0.16^{c}$ | $45.02 \pm 0.16^{e}$ | $5.67 \pm 0.49^{ef}$ | 45.02 |
| *Hibiscus* sp. | $7.58 \pm 0.27^{b}$ | $12.35 \pm 0.22^{g}$ | $33.30 \pm 0.69^{d}$ | $6.37 \pm 0.24^{f}$ | 33.30 |
| *Hibiscus cannabinus* | $7.58 \pm 0.33^{b}$ | $14.13 \pm 0.09^{h}$ | $25.97 \pm 0.36^{c}$ | $5.04 \pm 0.17^{e}$ | 25.97 |
| *Hibiscus surratensis* | $3.96 \pm 0.40^{a}$ | $12.83 \pm 0.57^{g}$ | $28.15 \pm 1.49^{c}$ | $2.53 \pm 0.23^{bc}$ | 28.15 |
| *Ipomoea eriocarpa* | $10.41 \pm 0.78^{d}$ | $4.07 \pm 0.11^{a}$ | $64.25 \pm 0.54^{f}$ | $1.59 \pm 0.30^{a}$ | 64.84 |
| *Maerua angolensis* | $4.81 \pm 0.28^{a}$ | $10.20 \pm 0.14^{e}$ | $17.04 \pm 0.35^{b}$ | $2.25 \pm 0.25^{abc}$ | 17.04 |
| *Senna obtusifolia* | $8.95 \pm 0.15^{c}$ | $14.94 \pm 0.08^{i}$ | $27.93 \pm 1.22^{c}$ | $2.83 \pm 0.31^{c}$ | 27.93 |
| *Vigna membranacea* | $4.06 \pm 0.43^{a}$ | $4.67 \pm 0.18^{ab}$ | $18.89 \pm 0.50^{b}$ | $2.82 \pm 0.20^{c}$ | 18.89 |
| Mean square | 15.2 | 44.6 | 673.0 | 7.5 | |
| *F*-statistics at $df = (1,11)$ ($p < 0.001$) | 103.3 | 946.9 | 1106.1 | 93.68 | |

GAE, mg/g: milligram of gallic acid equivalents per gram of dry residue, RE, mg/g: rutin equivalent per gram residue, $IC_{50}$ of ascorbic acid = 12.82 ppm. Values are expressed as means ± SD ($n = 3$). Means in columns followed by different letters indicate significant difference between different plant species at the $p < 0.05$ levels by Tukey HSD *post hoc* test.

regarded as anti-nutritional factors in foods (61), they are also known to have anti-inflammatory, wound healing (62) and antibacterial properties (63) and have remarkable ability in prevention of cancer (64, 65). Therefore, wild edible plant species containing this compound may be a potential bioactive compound source in cancer treatment.

The flavonoid content of the plant species varied from 4.07 ± 0.11 in *I. eriocarpa* to 14.94 ± 0.08 RE, mg/g in *S. obtusifolia*.

The total flavonoid contents in this study is similar to those reported for similar species in previous works (66) on selected Ugandan medicinal plant foods, i.e., 12.0 ± 0.2 for *S. obtusifolia* and 12.9 ± 1.0 for *Hibiscus* sp. In contrast, other studies have shown higher flavonoid content of wild edible plants compared to ours. For example, Lamien-Meda et al. (67) showed the flavonoids content of 14 wild edible fruits of Burkina Faso to vary from 1.70 ± 0.35 to 116.05 ± 3.04 RE, mg/g. Andabati and Muyonga (66) studied selected Ugandan traditional medicinal foods, and indicated higher flavonoid content in *H. cannabinus* (38.4 ± 0.9 RE, mg/g) and *I. eriocarpa* (78.9 ± 2.7) than in the present study. Yang et al. (68) recorded lower total flavonoids contents of 2.54 mg/g fresh weight in wild edible plants of the World Vegetable Center-southern Taiwan. Flavonoids confer characteristic tastes in foods, thus promoting peculiar tastes in prepared foods. Naturally, flavonoids are reported to have a wide range of beneficial effects on humans and are therapeutically potent against a wide range of diseases (69, 70). They exhibit their actions through effects on membrane permeability and by interfering in enzyme activity and scavenging free radicals (71, 72).

The content of total phenolic compounds in this study was high and ranged from of 13.39 ± 0.26 in *A. rhomboidea* to of 64.25 ± 0.54 mg GAE/g dry matter in *I. eriocarpa*. A previous study by Andabati and Muyonga (66) has also reported higher amount (91.9 mg GAE/g) of phenolic compounds in *I. eriocarpa.* The obtained results corresponded well with the range of the values previously reported in some other studies (e.g., (67)). According to Lamien-Meda et al. (67), the total phenolic content of the methanol extracts from some wild edible plants from Burkina Faso varied from 1.91 to 49.47 GAE, mg/g, which is comparable to those in the present study. Meanwhile, our study recorded total phenolic content lower than that of Yang et al. (68) on Argentinean wild grapefruits (320 GAE, mg/g) and Chilian variety of 117 GAE, mg/g. The variation of phytochemical contents in the wild edible plants could be attributed to the difference in plant species, environmental conditions (73), plant parts used (74) and the solvent used for extraction (75). Besides, polyphenolic compounds are a key component of all the plant-derived foods and these act primarily as antioxidants, and ant-inflammatory agents (62). Phenolic substances also have antiseptic properties (15).

The alkaloids content varied from 1.59–6.37 ± 0.24 g/100 g and it differed significantly among the wild edible plants. Some vegetables like *C. ochroleuca* are bitter due to the presence of alkaloids belonging to the quinolizidines group (76). Some alkaloids possess medicinal properties such as amoebicidal and antitumor activity (77, 78).

The results of DPPH scavenging activity assay indicate that these wild edible plants are potentially good sources of natural antioxidants. In this study, the free radical scavenging activity determined by DPPH varied from13.39 to 64.84 μg/ml, which was comparable to values (0.1 to 57.8) obtained in selected Ugandan traditional medicinal foods from Kamuli to Gulu districts (66). For example, the antioxidant activities of *H. cannabinus* and *C. ochroleuca* were 22.2 ± 1.8 and 8.8 ± 0.7 milligram ascorbic acid equivalents per gram dry weight (mg VCE $gDW^{-1}$), compared to 25.97 and 13.72 μg/ml in this study. However, the antioxidant potential of the 12 wild edible plants is not as effective as DPPH radical scavengers when compared to ascorbic acid. This is contrary to other findings that reported a higher antioxidant capacity of plant extracts than ascorbic acid (79, 80). The low oxidative potential could be attributed to the extraction solvents used for each study (81, 82). Nonetheless, our results are comparable to the values of DPPH radicals scavenging activities of Brazilian wild medicinal plants (10 ppm), as reported by Brighente et al. (83); which was also lower than that of the ascorbic acid standard (8.4 ppm). This variation in antioxidant activities could be attributed to differences in the wild edible plant species studied, climatic conditions and soil types (84). However, one major limitation of this study was that the antioxidant activity was described by only one method, yet, antioxidant activity can be influenced by many factors and because of the complex nature of many plants and differences in the mechanism of action of antioxidants (85). Nevertheless, the highly significant and positive correlation of the DPPH radical scavenging $IC_{50}$ with total tannins and total phenolic content could imply that tannins and phenols are the main contributors to the radical scavenging activity of the selected plants. A high correlation between the content of phenolic compounds and the scavenging effect of DPPH has also been previously demonstrated by Noreen et al. (86) in study from Pakistan and by Andabati and Muyoinga (66) in wild edible plants of Gulu and Kamuli districts in Uganda. Several factors might influence antioxidant activity and, therefore might not be fully described by a single assay. A reliable antioxidant evaluation protocol could have required employing different antioxidant activity assessment methods, such as nitric oxide radical scavenging, ferric reducing power and total antioxidant assays. Given that only one method was employed in this study, this can be a limitation of the study.

## Conclusion

This study shows that the leaves of wild edible plants consumed by the Acholi communities in northern Uganda contain a wide range of bioactive phytochemicals and are a rich natural source of antioxidants. The highest contents of both total phenolic and tannins were found in *I. eriocarpa*. *Senna obtusifolia* and *Hibiscus* sp. presented the highest contents of total flavonoid and total. Among the selected edible plants, *A. rhomboidea* provided the highest activity for antioxidants. Hence their regular consumption could provide human health benefits by protecting against oxidative stress related diseases. The high correlations confirm the roles of phenol and tannin compounds as the main contributor to the antioxidant activities of these plants. Further investigations are needed to characterize the phytochemicals in the wild edible plants, profiling the plant extracts with high antioxidant activity by LC–MS and determining their microbial activities. This will stimulate interest in wild edible plant use in the nutraceutical industries and new drug development.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

AN, GM, and GA designed this study. AN collected, analyzed samples, and wrote the initial draft of the manuscript. GM, IA and GA were responsible for data interpretation and editing of the manuscript. 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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# An evaluation of nutrition, culinary, and production interventions using African indigenous vegetables on nutrition security among smallholder farmers in Western Kenya

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**Introduction:** Nutrition security continues to worsen in sub-Saharan Africa. Current research is limited on how seasonality may influence the impact of nutrition, culinary, and production interventions on food security, diet quality, and consumption of African Indigenous Vegetables (AIV); a culturally accepted source of micro-and-macronutrients that are easily produced due to their adaptation to the local environment. The objective of this study was to evaluate the programmatic impact of AIV interventions on nutrition security among smallholder farmers.

**Methods:** In a randomized control trial, five target counties in Western Kenya were randomly assigned to one of four treatments: (1) control; (2) production intervention (PI); (3) nutrition and culinary intervention (NCI); and (4) NCI and PI (NCI/PI). After the counties were randomly assigned to a treatment, 503 smallholder farmers (18–65 years) were selected from participatory farmer groups. The PI consisted of five agricultural production modules delivered between 2016 and 2019. The NCI was delivered twice: (1) household nutrition education (2017) and (2) community culinary training (2019). The NCI/PI included communities receiving both interventions at these time periods. Baseline and endline surveys were administered to all participants once in October 2016 (harvest season) and to all available participants ($n$=250) once in June to July 2019 (dry season), respectively. The impact evaluation was analyzed by Household Hunger Scale (HHS), Women's Dietary Diversity Score (WDDS), AIV consumption frequency, and AIV market availability. Statistical tests included descriptive statistics (means and frequencies), paired $t$-test, McNemar's test, Wilcoxon Signed-Rank test, ANOVA test with Tukey *post hoc*, and $\chi^2$ test. Open-ended questions were aggregated, and responses were selected based on relevancy and thoroughness of the response to provide context to the quantitative data. A value of $p$<0.05 was used to denote statistical significance.

**Results:** There was an overall decrease in WDDS, HHS, and consumption frequency between baseline and endline attributed to seasonal differences. Despite this, post-intervention, households that received NCI/PI had a higher WDDS relative to the control: WDDS 5.1±1.8 vs. 4.2±1.5, $p$=0.035. In addition, between baseline and endline, there was an overall increase in the percentage of respondents that reported an adequate supply of key AIVs, particularly for households that received PI. Furthermore, seasonal effects caused a reported shift in the primary location for purchasing AIVs from the village to the town market. There was no reported difference in HHS. While "diet awareness" significantly influenced diet quality among the NCI treatment group, "production" was reported to have the greatest influence on diet quality among all intervention groups.

**Discussion:** The findings revealed that coupled nutrition, culinary, and production interventions could create a protective effect against seasonal fluctuations in the availability and affordability of AIV as evidenced by a higher WDDs.

**Conclusion and Recommendations:** These findings suggest that future programming and policy should focus on promoting the availability, accessibility, acceptability, and affordability of improved agronomic practices and germplasm for both smallholder farmers with particular emphasis on AIV varieties that contain high levels of micro-and macronutrients, improved agronomic characteristics (e.g., delayed flowering, multiple harvests, higher yields, and disease resistance), and are aligned with the communities' cultural preferences. In addition, agricultural training and extension services should incorporate nutrition and culinary interventions that emphasize the importance of farmers prioritizing harvests for their household consumption.

KEYWORDS

agriculture, behavior change, cooking skills, healthy diets, malnutrition, micronutrients, traditional vegetables, orphan crops

## 1. Introduction

Food security continues to worsen in sub-Saharan Africa (SSA). The number of undernourished or hungry people in Africa, defined by FAO as those who consume an insufficient number of calories over the course of a year, increased by 46 million between 2019 and 2021 (1). In addition, inadequate intake of micronutrients, such as iron and zinc, are widespread in SSA (2), with half of all anemia cases resulting from dietary iron deficiency (3). The prevalence of undernutrition, which had remained stagnant for many years, worsened due to the COVID-19 pandemic. More specifically, in Kenya, the prevalence of moderate or severe food insecurity in the total population increased from 53% in 2014–2016 to 68.5% in 2018–2020 (1). Poor diets and subsequent inadequate caloric and nutrient intake increases the risk of infectious diseases and micronutrient deficiencies, especially vitamins A and iron, which pose major impediments to social and economic development (1). To improve these diet-related outcomes, it is necessary to design food systems that improve dietary quality and reduce the prevalence of nutrition insecurity, by increasing total energy (calories from macronutrients) and micronutrient intake. Moreover, food system shocks, such as the COVID-19 pandemic are expected to worsen under climate change. Recognizing this major challenge, USAID developed a multi-sectoral nutrition strategy for 2014–2025 to decrease malnutrition, improve nutrition and increase economic productivity (4).

African Indigenous (traditional) Vegetables (AIVs) have unmet potential to contribute to economic and human health in SSA. AIVs are vegetables that either originated or have a long history of cultivation and domestication in Africa and are locally important for economic and human nutrition but have yet to gain regional and global recognition as a major commodity such as carrots or corn (5, 6). AIVs such as African nightshade (*Solanum scabrum*), amaranth (*Amaranthus* spp.), cowpea leaves (*Vigna unguiculata*), and spider plant (*Cleome gynandra*) are culturally accepted through custom, habit, or tradition (7–12) and nutritionally dense (13, 14). In addition, these plants are adapted to the local environmental conditions and often exhibit tolerance to extreme temperatures and precipitation allowing them to be sustainably produced with little to no inputs (8, 15, 16). The combination of these attributes positions AIVs as a rich micro-and macronutrient, climate-resilient food source with unmet economic potential (11). Despite this, limited seasonal availability, poor market access, and high prices impede local production and regular household consumption (8, 9, 17). These difficulties can be overcome by focusing agricultural interventions, programming, and policy on promoting the availability, accessibility, acceptability, and affordability of improved agronomic practices and AIV germplasm, prioritizing high levels of micro-and macronutrients and improved agronomic characteristics (e.g., delayed flowering, multiple harvests, higher yields, and disease resistance) for smallholder farmers. In addition, agricultural training and extension services should incorporate nutrition and culinary interventions that emphasize the importance of farmers prioritizing harvests for their household consumption to ensure nutrition security.

Nutrition security, defined as having consistent access, availability, and affordability of foods and beverages that promote well-being and prevent (and if needed, treat) disease, is required to improve diet-related health outcomes (18). Education on healthy nutrition, good eating habits, food preparation, and safe handling are among effective strategies for overcoming malnutrition and chronic diet-related diseases, such as obesity, diabetes, hypertension, and cardiovascular diseases (19–22). In addition, agricultural interventions that promote the production of nutritionally dense foods such as AIVs can improve access, and availability, and increase household income either through generating income from the sale of produce or saving income from food expenditures (11, 23–25). Current research and literature, however, is limited on how seasonality may influence the impact of nutrition, culinary, and production interventions on nutrition security, diet quality, and consumption of AIVs among smallholder farmers in Western Kenya.

Through a randomized control trial (RCT), this study aimed to evaluate the programmatic impact of a nutrition, culinary, and production interventions on nutrition security during the dry season, in comparison to the harvest season, among smallholder farmers in Western Kenya. We hypothesized that overall diet quality and consumption of AIVs will decrease during the dry season; however, we also hypothesized that the treatment groups would have improved dietary outcomes relative to the control.

# 2. Methods

## 2.1. Overview of study setting

This study was part of a larger research initiative to examine the production and consumption of AIVs in Kenya supported by the USAID Feed the Future Innovation Laboratory for Horticulture (9–11, 26). This study engaged five counties in Western Kenya: Kisumu, Nandi, Busia, Bungoma and Trans Nzoia Counties. Kisumu and Nandi County were treated as one unit due to their proximity to Kisumu City (large market access for AIVs). Agriculture is the main economic activity in the study counties (27, 28). The staple food crop, maize, is often consumed as stiff porridge (*ugali*) alongside cooked leaves of AIVs (29). Moreover, the intervention communities had prior exposure to African Indigenous Vegetables (AIVs) innovation programs and training through USAID-funded Horticulture Innovation Programs (12).

## 2.2. Intervention design

The study objective was to evaluate the impact of a production, nutrition, and culinary intervention on nutrition security among smallholder farmers in Western Kenya. To that end, the four treatment areas were randomly assigned to one of four interventions summarized below. Figure 1 provides an overview of the program interventions and participant selection.

1. *Control*: No additional intervention or agricultural training.
2. *Production Intervention* (*PI*): Communities received the production intervention, which addressed key bottlenecks in production and distribution, including cultural practices, management, and technologies, improved seed, integrated pest management, irrigation, and drought tolerance management.
3. *Nutrition and Culinary Intervention* (*NCI*): Communities received a two-part nutrition and culinary intervention: (1) promotion of AIV consumption in the households through nutrition education; and (2) community level culinary training. Topics included recommended daily-intake guidelines, recipe and meal-preparation, data on nutrition for each AIV, bodily processes supported, and symptoms of malnutrition alleviated.
4. *PI and NCI:* Communities received both the production and nutrition and culinary interventions.

The five production intervention modules were hosted at demonstration farms central to the intervention communities between October 2016 and May 2019. The modules were delivered by project partners at the Academic Model Providing Access to Healthcare (AMPATH) in Eldoret, Kenya. Each module was delivered within a half to full day session at the demonstration farm. The modules were designed using Good Agricultural Practices evidenced-based best practices developed by the Economic Empowerment and Agricultural division of AMPATH. After each production module was delivered, the trainers would return to the farming community to provide additional support.

The two-part nutrition and culinary intervention were delivered by study members from Kenya Agricultural and Livestock Research Organization (KALRO) and AMPATH as well as locally trained community health workers. Before the nutrition and culinary intervention was delivered, each component was piloted with neighboring communities. July 2017, the first nutrition and culinary intervention was administered at the household level with one or both heads of household. The intervention used a nutrition and culinary pamphlet that was designed from insights gained during a series of focus group discussions with households in the target communities (9). In May 2019, the second nutrition and culinary intervention was delivered at the community level. The intervention households were grouped into clusters of 20, based on location and invited to participate in a community cooking day. As requested by the study community (9), the intervention focused on improved cooking methods and further developing cooking skills such as cooking dried AIV leaves, reduced cooking time, and mixing ingredients.

## 2.3. Sampling and randomization

Before the start of the project, five counties were purposively selected for implementation of the interventions based on prior work with the community groups by project team members. Each of the study county or cluster of counties was randomly assigned to one of the four treatments. We obtained lists of 53 farmer groups and lists of group members from intervention implementation partner (AMPATH; Table 1). All members of the participating farmer groups were allowed to participate in the interventions; however, only a subset participated in the baseline and endline surveys. The lists of group members formed the sampling frame from which, 5–12 individual members were randomly sampled proportionate to the group sizes for inclusion in this study ensuring that the study was representative among smallholder farmers in Western Kenya. The group members were assessed for inclusion eligibility based on the following criteria: the group member was from a household had a primary farmer, male or female aged 18–65 years, and owned a small farm or garden (defined as <1 ha). In addition, the household had to have a woman over 18 years to participate in the Women's Dietary Diversity questionnaire. Exclusion criteria include any households

TABLE 1 Assignment and number of households to the four intervention treatments in Western Kenya.

| | | | Number of surveyed households | |
|---|---|---|---|---|
| Treatment | County | Sub-county(s) | Baseline (2016) | Endline (2019) |
| Control | Trans Nzoia | Kiminini | 126 | 61 |
| Production intervention | Nandi | Nandi South | 41 | 20 |
| | Kisumu | Kisumu West | 85 | 44 |
| Nutrition and culinary intervention | Bungoma | Sirisia, Webuye East, and Webuye West | 126 | 56 |
| Production and Nutrition/culinary intervention | Busia | Matayos and Teso South | 125 | 69 |
| Total (*n*) | | | 503 | 250 |
| Attrition *n* (%) | | | | 253 (50) |

that were horticultural farmers or commercial farmers cultivating/managing land more than 3 ha regardless of whether they had a small kitchen garden. Figure 2 provides an overview of the timeline of the program interventions.

A total of 503 households, across counties, were randomly selected using a random sampling procedure from farmer group members' lists to participate in the baseline survey before roll-out of the project interventions. At endline, 250 households, across counties (Figure 2), who were interviewed during the baseline survey also participated in the endline survey. The attrition between baseline and endline surveys was mainly attributed to respondents not being found at home at the time of the survey for several reasons despite at least one repeat visit or appointment. The main reasons included involvement of potential respondents in off-farm businesses, attending social functions such funerals and community meetings, separation or divorces in the households, migration, fall-out of members from their groups and death. A high attrition was anticipated due to instability in the rural area and potentially difficulty access communities due to extreme weather (30, 31). No incentives or gifts were given to any of the treatment or non-treatment groups for participation in the survey or research. However, after the intervention, a small quantity of AIV seeds were given to all households who had participated in the surveys.

## 2.4. Survey instrument

The baseline and endline structured questionnaire were developed by Rutgers University, United States in collaboration with KALRO in English and translated into the local languages. The questionnaires contained nine sections: (1) identifying respondents and the study area; (2) household demographics; (3) household living conditions; (4) household general food consumption frequencies; (5) consumption and utilization of AIVs; (6) a modified FANTA III Household Hunger Scale (HHS) (32); (7) consumer attitudes and preferences; (8) FANTA III Women's Dietary Diversity Score (WDDS) (33); and (9) women's role in decision making. The endline survey contained one additional section: participant feedback. This section ascertained data relative respondent's perception of their dietary changes and the reasons for these changes post-intervention, with the later questions being open-ended. A copy of the endline survey can be found in Supplemental material Appendix A.

Due to time constraints, for HHS, this study used a modified FANTA III. The survey focused on three questions [(1) no food of any kind, (2) go to sleep hungry, and (3) go a whole day or night without food] and their subsequent frequency questions. HHS was then calculated for each respondent by summing the points relative to each response. Using the following scores (e.g., 1 for yes; 1 for "sometimes," and 2 for "often") each question had a maximum of three points for a total HHS of 0 (low household hunger) to 9 (high household hunger).

For WDDS and AIV consumption, at an individual level, the eldest female in the household was asked whether they consumed numerous food groups, from an extensive list of food groups and specific foods (e.g., different AIVs) on a regularly basis within a 24-h period. The food groups were then aggregated into the following nine categories used to calculate WDDS: starchy staples; dark green leafy vegetables; other fruits and vegetables; organ meat; meat and fish; eggs; legumes, nuts, and seeds; and milk and milk products. WDDS was calculated by summing the total number of food groups consumed from 0 (low dietary diversity) to 9 (high dietary diversity) (33). In addition, AIV consumption was calculated by summing the total number of AIVs consumed from 0 (low AIV consumption diversity) to 11 (high AIV consumption diversity).

Reported food frequencies were recoded as follows: never = 0, sometimes = 1, once a month = 0.25, and everyday = 7. The questionnaire ascertained data relative to food consumption frequency for 28 different foods with a possible total of seven points per food for a total of 196 points. Post-intervention, the following food frequency tertials were calculated to group the respondents: low = 0–29.25; middle = 29.26–36.25; and high = 36.36–196.

## 2.5. Data collection

Each participant included in the study completed a total of two surveys: (1) baseline and (2) endline. The baseline and endline survey were conducted in October 2016 (wet season) and in June and July 2019 (dry season), respectively. Data were collected through face-to-face interviews by a team of locally recruited and trained enumerators. Prior to the interview, all potential respondents were made aware of the purpose of the survey as well as the overall objective of the study. The questionnaire was administered in *Kiswahili, Luhya,* and *Luo* dialects. The implementation of field data collection was overseen by lead KALRO collaborating agricultural economist, who monitored completeness and consistency of the responses to ensure that all sections of the questionnaires had been answered appropriately.

The data were validated by the lead agricultural economist and uploaded to the server and downloaded in Excel Software for ease of management and analysis on a daily basis. The data were matched with the baseline data using the same household code. Data were cleaned and exploratory checking conducted to identify key anomalies and outliers. The data were anonymized using codes of respondents, which were only known to lead economist. For confidentiality of respondents, data with no identifiers were shared with Rutgers University collaborators for analysis.

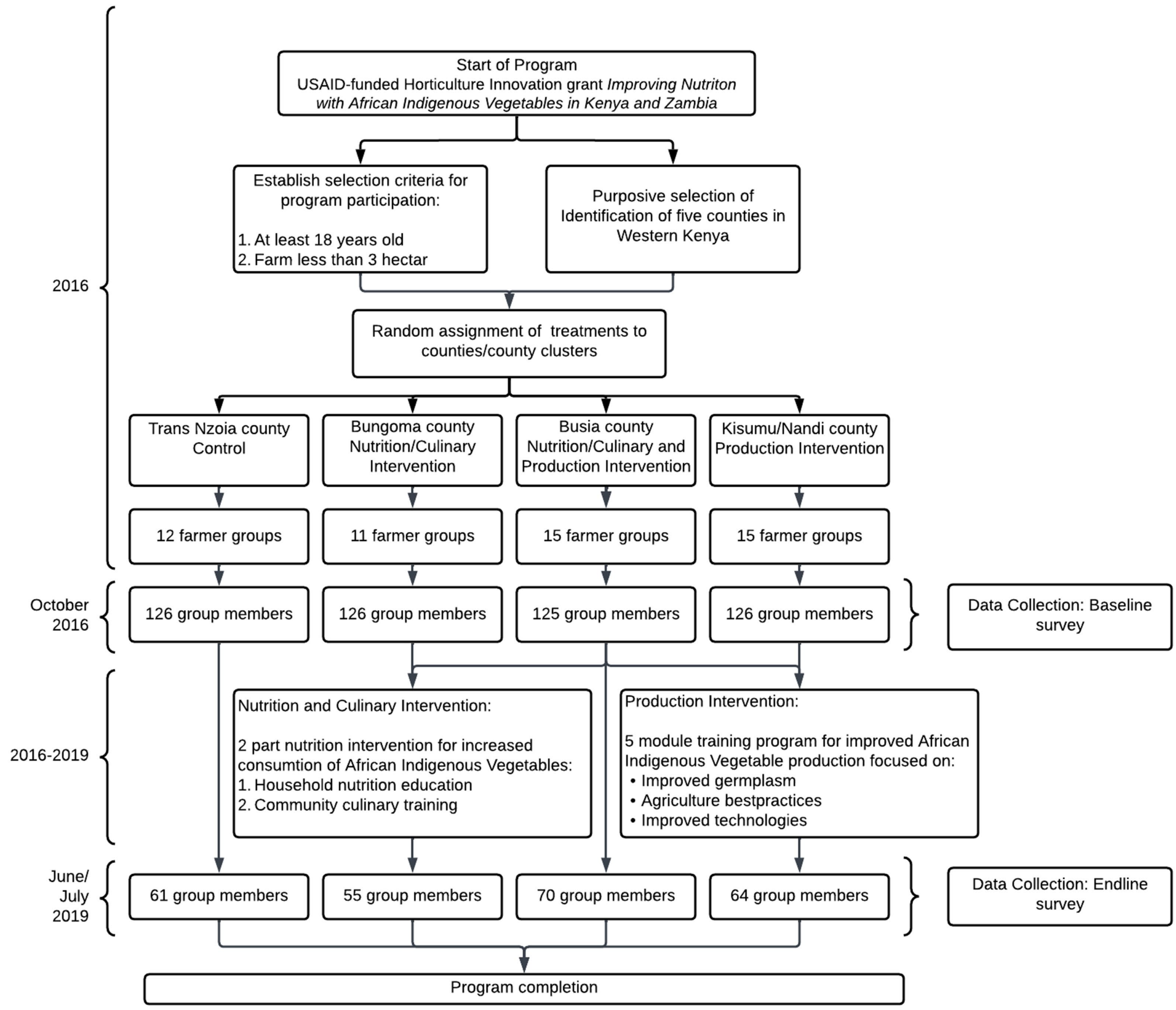


FIGURE 1
Participant flow diagram.

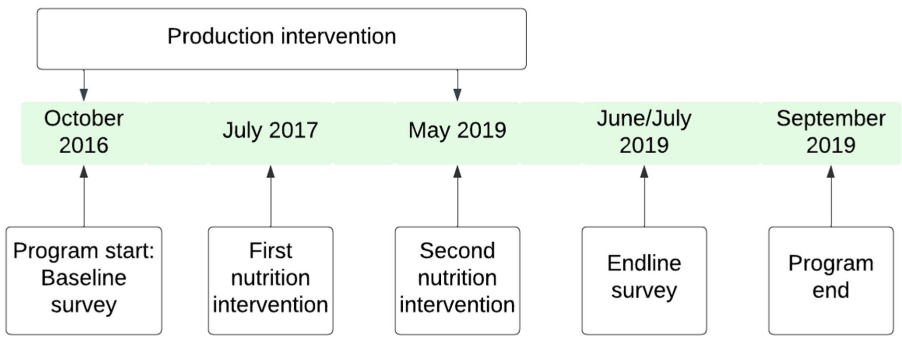


FIGURE 2
Program timeline.

Data were collected by 14 enumerators. The enumerators were selected based on educational background, prior experience, knowledge of the local language, and familiarity with the areas where data collection was to occur. A total of 14 enumerators were trained at a central venue where they were taken through the content of the questionnaire. Additional training topics included understanding the objectives of research, understanding questionnaire content, framing of questions, and field implementations and procedures. Additionally, the enumerators and those handling the data were each trained and received *Collaborative Institutional Training Initiative* (*CITI* Program) certification prior to survey initiation.

Before embarking on the final data collection, the enumerators and the supervisors pretested the questionnaire with households near the training venue (Busia Agricultural Training Center) to ensure that the enumerators fully understood the content of the questionnaire, the order of the questions, and the skip patterns. At endline, the questionnaire was programmed in Open Data Kit (ODK) platform and loaded in the mobile phones. Enumerators received additional training on the use of mobile phones and applications for data collection.

## 2.6. Data analysis

Descriptive statistics (means and frequencies) were used to summarize respondent data. A paired *t*-test and a McNemar's test was used to examine the statistical differences, either in continuous or in categorical data respectively, in means between timepoints within the same sample population (e.g., baseline and endline analysis per treatment group). A Wilcoxon Signed-Rank test was used to examine the statistical differences in means for WDDS due to a lack of normal distribution.

Using only the post-intervention data, an ANOVA test was used to examine the differences in means and treatment groups. A Tukey *post hoc* ANOVA test was used to determine the statistical differences between groups. In addition, post-intervention, a $\chi^2$ test was used to calculate the statistical differences between categorical data and treatment groups. Open-ended questions were aggregated, and responses were selected based on relevancy and thoroughness of the response to provide context to the quantitative data.

Pairwise deletion was used to handle cases of missing data. Quantitative analyses were conducted using SPSS (IBM SPSS Statistics v 26; Armonk, New York, United States) and a *p* value of <0.05 was used to denote statistical significance.

## 2.7. Ethical considerations

Ethical approval in the United States was provided by the Institution Review Board at Rutgers University, the State University of New Jersey. Ethical approval in Kenya was provided by the Institutional Research and Ethics Committee at the Academic Model for Providing Access to Healthcare (AMPATH) in Kenya. Before beginning the interview, the enumerators introduced themselves and assured participants that the survey was voluntary and that there would be no disclosure of individual information. All study participants provided informed oral consent to participate in the study and for use of the data in publications. The use of oral consent was approved by the ethical review boards due to minimal associated risk and low literacy rates among the study population.

# 3. Results

## 3.1. Demographic

Due to program attrition, a total of 250 households were included for analysis. Table 2 provides an overview of the demographic characteristics of the study population. Most households had both male and female heads of households and male heads of households were an average 51.1 years old. In addition, the majority of heads of households were married and both male and female heads of household had primary education. Households had an average size of seven members, had three sources of income, and consumed three meals per day; however, the control group reported consuming significantly more meals compared to PI and NCI/PI intervention groups at baseline ($p = 0.001$ and $p = 0.005$ respectively).

## 3.2. Intervention influence on diet quality

Overall, there was a significant decrease in dietary diversity ($p < 0.001$) and an observed decrease in other outcome variables post-intervention (Table 3). Post-intervention, the NCI/PI intervention group reported a significantly higher WDD score relative to control: 5.1 ± 1.8 vs. 4.2 ± 1.5, $p = 0.035$. However, this group also reported a significantly higher household hunger score post intervention relative to control: 0.9 ± 1.5 vs. 0.3 ± 1.0, $p = 0.05$. The intervention groups that received the nutrition intervention (NCI and NCI/PI) reported the highest proportion of participants whose food frequency score was within the lowest tertial. In addition, the PI intervention group reported the highest proportion of participants whose food frequency score was within the middle tertial.

At endline, there was a significant difference in consumption patterns between the intervention groups (Figure 3). The NCI and NCI/PI intervention groups, respectively, reported a significantly higher percentage of respondents consuming the following food groups and AIVs within a 24-h period relative to control (Figure 4): dark leafy greens (51 and 66% vs. 36%, $p = 0.003$), organ meat (7 and 16% vs. 3%, $p = 0.016$), eggs (29 and 40% vs. 10%, $p = 0.001$), spider plant (36 and 40% vs. 15%, $p = 0.004$), and jute mallow (27 and 33% vs. 8%, $p = 0.007$). Moreover, all intervention groups reported consuming significantly more cowpea relative to control ($p = 0.001$); however, NCI group reported consuming significantly less Ethiopian mustard relative to the other treatment groups ($p = 0.05$).

## 3.3. Source and supply of food items post-intervention treatment

Prior to the intervention, respondents reported sourcing the highest number of food items from their own or other farm (Figure 5). Post-intervention, there was a significant shift in locations used to source food items, with a decrease in all reported locations except village market. Post-intervention, respondents reported acquiring over five items, on average, from the village market, compared to less than one at baseline ($p < 0.001$). Moreover, there was a significant increase in individuals reporting sourcing food items from the city market ($p < 0.001$).

Post-intervention there were differences in the percentage of respondents who reported an adequate supply of various AIVs between treatment groups (Figure 6). There was a significant difference in reported supply for jute mallow ($p = 0.002$) and Ethiopian mustard ($p = 0.001$) between treatment groups. The highest percentage of respondents in both the NCI and PI intervention groups reported an adequate supply of jute mallow;

TABLE 2 The 2016 demographic information of the surveyed households in Western Kenya.

| | Control | NCI | PI | PI and NCI | All households | *p* value[1] |
|---|---|---|---|---|---|---|
| *N* | 61 | 55 | 64 | 70 | 250 | |
| County | Trans Nzoia | Bungoma | Kisumu and Nandi | Busia | | |
| Heads of household (HH) (%) | | | | | | |
| *Only male* | 3.3 | 12.7 | 18.8 | 14.3 | 12.4 | 0.063 |
| *Only female* | 24.6 | 18.2 | 32.8 | 17.1 | 23.2 | 0.133 |
| *Both male and female* | 72.1 | 69.1 | 46.9 | 68.6 | 64.0 | 0.011* |
| Male HH Age (mean ± SD) | 55.5 ± 11.7 | 52.8 ± 13.0 | 48.2 ± 13.4 | 48.7 ± 15.5 | 51.1 ± 13.8 | 0.034* |
| HH marital status (%) | | | | | | 0.149 |
| *Married* | 83.6 | 81.8 | 68.8 | 81.4 | 78.8 | |
| *Widowed* | 13.1 | 14.5 | 26.6 | 11.4 | 16.4 | |
| *Other* | 3.3 | 0 | 1.6 | 4.3 | 2.4 | |
| *Missing* | 0 | 3.6 | 3.1 | 2.9 | 2.4 | |
| HH education (%) | | | | | | 0.010* |
| *None* | 3.3 | 5.5 | 4.7 | 4.3 | 4.4 | |
| *Primary* | 41.0 | 50.9 | 54.7 | 65.7 | 53.6 | |
| *Secondary* | 39.3 | 43.6 | 29.7 | 25.7 | 34.0 | |
| *University and higher* | 14.8 | 0 | 7.8 | 0 | 6.0 | |
| *Missing* | 1.6 | 0 | 3.1 | 2.9 | 2.0 | |
| HH spouse education (%) | | | | | | 0.004* |
| *None* | 0 | 5.5 | 4.7 | 7.1 | 4.4 | |
| *Primary* | 39.3 | 45.5 | 53.1 | 60 | 50.0 | |
| *Secondary* | 41.0 | 34.5 | 14.1 | 18.6 | 26.4 | |
| *University and higher* | 1.6 | 0 | 6.3 | 1.4 | 2.4 | |
| *Missing* | 18.0 | 14.5 | 21.9 | 12.9 | 16.8 | |
| Household size (mean ± SD) | 6.9 ± 2.8 | 6.5 ± 1.9 | 6.4 ± 2.8 | 6.5 ± 2.4 | 6.6 ± 2.5 | 0.695 |
| Sources of income (mean ± SD) | 2.9 ± 0.8 | 2.6 ± 0.8 | 3.0 ± 0.6 | 2.6 ± 0.8 | 2.8 ± 0.7 | 0.010* |
| Number of meals consumed prior 24 h (mean ± SD) | 3.4 ± 0.9 | 3.2 ± 0.7 | 3.0 ± 0.4 | 3.0 ± 0.7 | 3.1 ± 0.7 | 0.001* |

NCI, nutrition/culinary intervention; PI, production intervention.
[1]Continuous variables (e.g., age, household size) between sample populations were compared using one way ANOVA with Tukey post hoc and categorical variables were compared using chi square.
*$p < 0.05$.

TABLE 3 Baseline and endline study outcome variables.

| | Baseline | Endline | *p* value[1] |
|---|---|---|---|
| Women's dietary diversity score | 7.9 ± 1.5 | 4.6 ± 1.7 | < 0.001** |
| Household hunger score | 0.5 ± 1.4 | 0.6 ± 1.3 | 0.48 |
| Food frequency score | 37.0 ± 18.4 | 34.8 ± 11.6 | 0.099 |

[1]*p* value for women's dietary diversity score calculated using Wilcoxon Signed-Rank test and household hunger score and food frequency score calculated with paired *t*-test.
**$p < 0.01$.

however, the NCI group reported an inadequate supply of Ethiopian mustard. When respondents were asked the main source AIVs, respondents in the NCI/PI, PI, and control intervention groups noted a significant increase post-intervention to receiving AIVs as gifts ($p = 0.004$; $p = 0.016$; and $p = 0.004$ respectively; Figure 7). Moreover, when respondents were asked the main location for purchasing AIVs, all treatment groups reported a significant decrease in purchasing AIVs at the village market pre-intervention ($p < 0.001$ all groups) to the town market post-intervention ($p < 0.001$ all groups; Figure 8).

## 3.4. Perception of dietary change post-intervention treatment

Respondents reported perceptions on dietary changes post-intervention by treatment (Figure 9). Post-intervention a majority of respondents, across treatment groups, reported a perceived improvement in diet quality. There was significant difference among treatment groups ($p = 0.011$) who reported "diet awareness" as the reason for improving diet with the highest reporting from the NCI

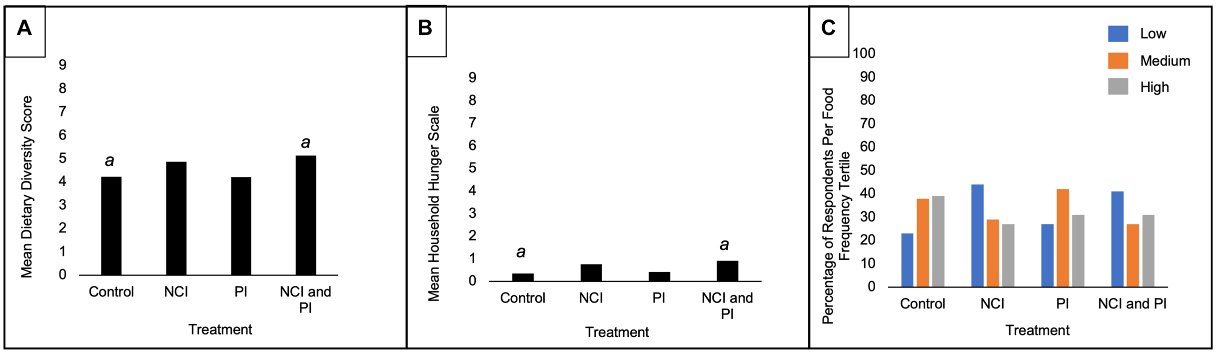


FIGURE 3
Post-intervention outcome variables by treatment interventions. NCI, nutrition and culinary intervention; PI, production intervention. [a]Statistically significant difference between treatments at $p<0.05$ (ANOVA Tukey *post hoc*). **(A)** Mean Women's Dietary Diversity Score. **(B)** Mean Household Hunger Score. **(C)** Percentage of respondents per food frequency tertial.

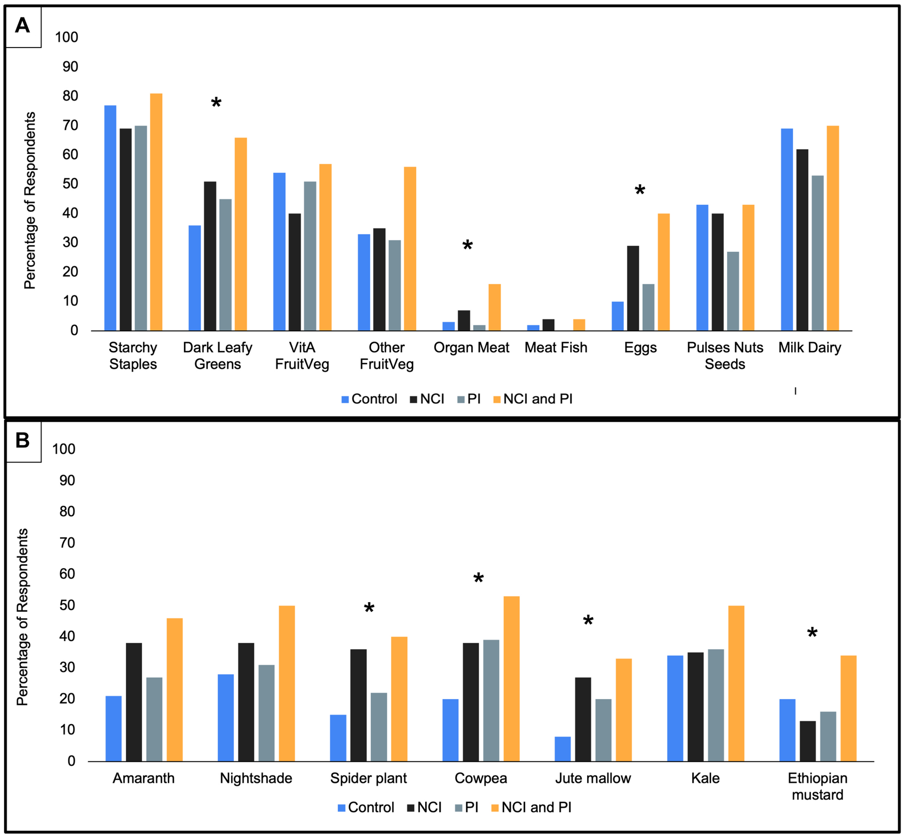


FIGURE 4
Food consumption by treatment interventions at endline. *Statistically significant difference between treatments at $p<0.05$ ($\chi^2$ test). **(A)** Food groups consumed in typical 24-h period. **(B)** African Indigenous Vegetables consumed in typical 24-h period.

treatment group. In addition, "production" and "income" were reported as reasons for improving diet quality. Moreover, regardless of treatment group, respondents noted that "change in production" had the greatest influence on diet. When respondents were asked to expound on the reasons that limited their ability to change their diet, they cited "lack of enough resources," "increased school fees," "loss of breadwinner," "lack of knowledge in preparing the vegetables in a better way," "knowledge," and "lack of money." When respondents were asked to explain reasons for their diet improvement "sufficient rain" and "few pests and diseases in crops" were reported.

## 4. Discussion

This study sought to examine the impact of nutrition, culinary, and production interventions on food security, diet quality, and AIV consumption in Western Kenya. Overall, there was a significant

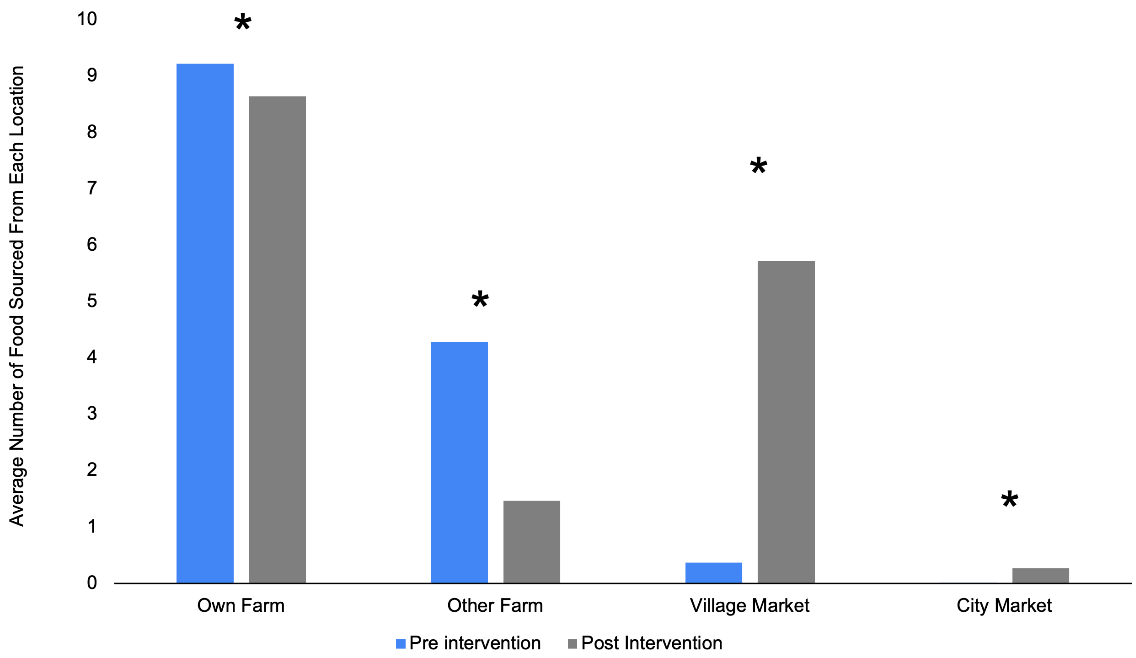


FIGURE 5
Average number of food items sourced from each location, overall, pre-and post-treatment interventions. *Statistically significant difference between baseline and endline at $p<0.05$ (paired $t$-test).

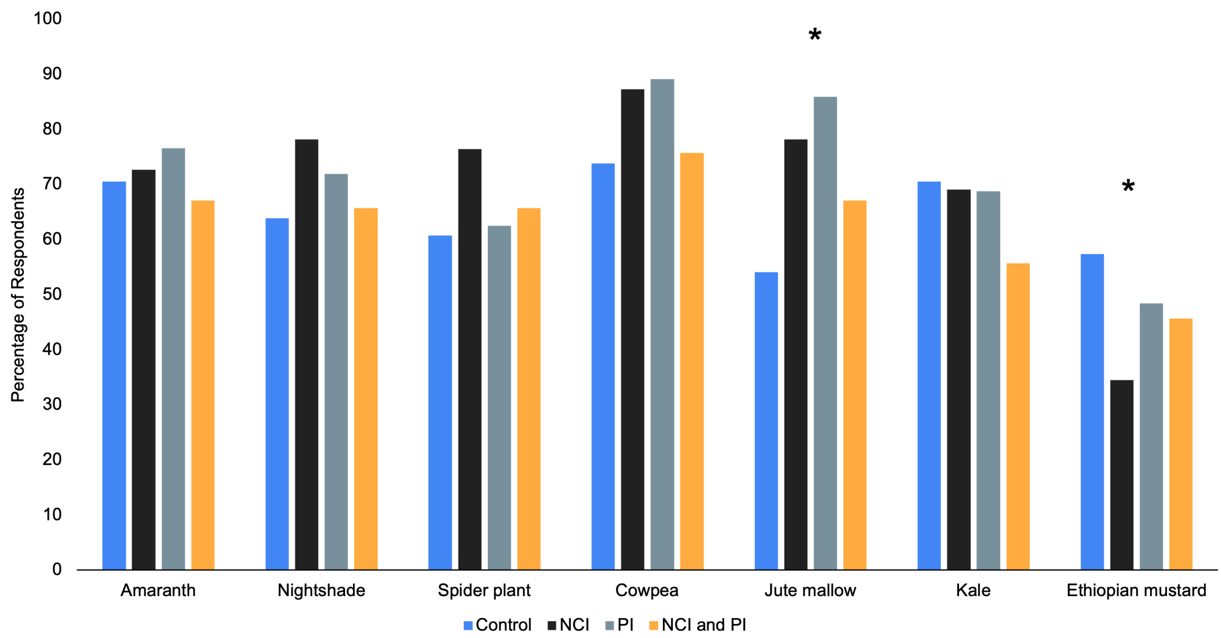


FIGURE 6
Percentage of respondents who reported an adequate supply of African Indigenous Vegetables post-intervention. *Statistically significant difference between treatments at $p<0.05$ ($\chi^2$ test).

decrease in WDDS, HHS, and consumption frequencies during the dry season. However, the nutrition, culinary, and production interventions posed some protective effect on diet quality. In addition, there was a reported shift in adequate supply and source location post-intervention. Despite a desire to change one's diet, and nutrition and culinary awareness, production remained the greatest influence on AIV consumption post-intervention.

## 4.1. Diet quality and AIV consumption patterns

Overall, seasonal differences resulted in an overall decrease in WDDS, HHS, and consumption frequency between baseline and endline. This coincides with existing literature where near daily consumption of AIVs was reported during peak seasons. However a

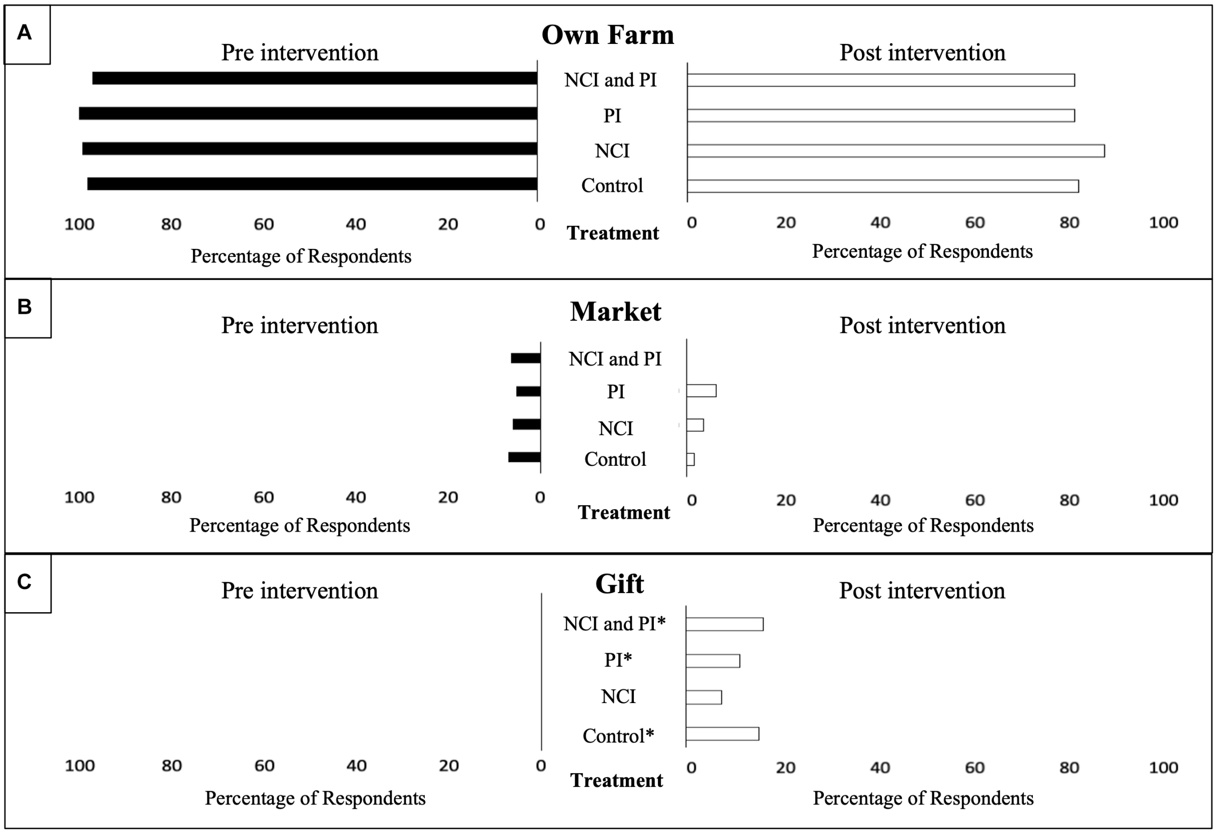


FIGURE 7
Reported supply of African Indigenous Vegetables (AIVs) by treatment pre-and post-intervention. *Statistically significant difference between baseline and endline at *p*<0.05 (McNemar's test). **(A)** Percentage of respondents sourcing AIVs from their own farm. **(B)** Percentage of respondents sourcing AIVs from the market. **(C)** Percentage of respondents sourcing AIVs as a gift.

decrease in consumption frequency as low as once a week was reported during off-seasons (34). Post-intervention, households that received both the nutrition/culinary and production interventions demonstrated a protective effect as measured by a higher WDDS relative to the control. Self-production, nutritional awareness, and necessary culinary skills gained in the NCI/PI intervention group could offset the reported limited availability and associated increase in market price reported during the dry season (35–37). Furthermore, income generated from selling surplus AIVs in the dry season could be used for household expenditures such as food (24). It is common for producers to favor sales over household consumption (38). This is further evidenced in this study by the significantly higher HHS in the NCI/PI intervention group relative to control. Therefore, it is important that interventions emphasize the importance of farmers prioritizing harvests for their household consumption.

In addition, in the NCI/PI intervention group, the reported high dietary diversity with corresponding high household hunger could be due to using a modified FANTA III survey to evaluate HHS. The HHS questions ascertained data relative to more severe household hunger (e.g., no food of any kind, go to sleep hungry, and go a whole day or night without food). While household members in this study area may have experienced these conditions within a 30-day period, on a typical day within a 24-h period, they may have also consumed a diverse diet leading to a high WDD score. Moreover, had the HHS questions ascertained data relative to low or moderate hunger such as whether household members ate smaller portions or ate food they would rather not eat, the data may have revealed different HHS scores in the different treatment groups.

## 4.2. Source and supply of food items

Post-intervention, respondents reported an overall increase an adequate supply of key AIVs, particularly for households that received the production intervention. AIV production is vulnerable to environmental stressors such as drought. However, this aggregate group of plants, which come from different plant families and thus differ genetically, do tend to be less sensitive to shocks as compared to "Western" introduced crops such as cabbage. This is also evidenced by each being found in the wild and successfully naturalized across many different environments. This positions AIVs as a climate-resilient commodity and stabilizes their availability throughout the year (8, 15, 39). In addition, the complementary agricultural training and continued extension support, which is often unavailable to smallholder farmers (40), could have strengthened their local value chain during the dry season. For example, a study in Vietnam by Sattaka et al. (41) reported that agricultural extension services influenced the production of culturally preferred glutinous rice and

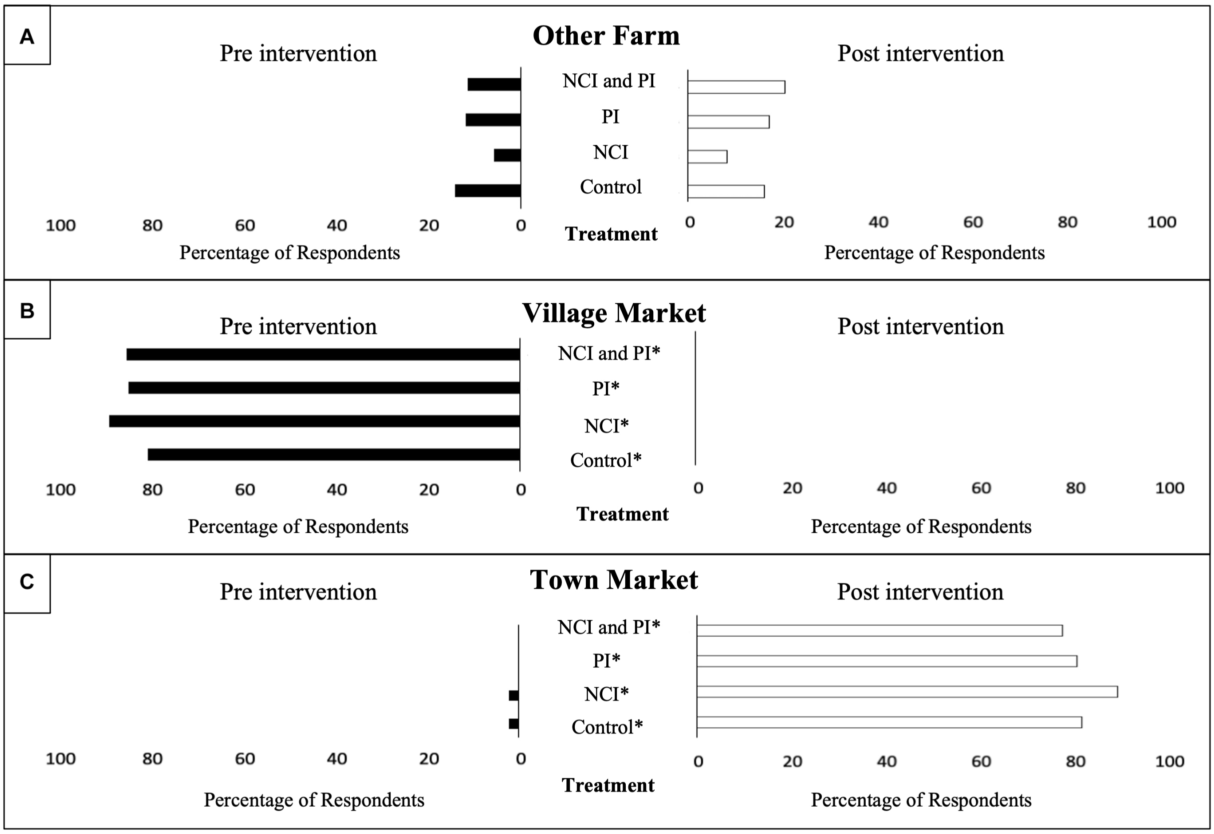


FIGURE 8
Purchasing place of African Indigenous Vegetables (AIVs) by pre-and post-treatment interventions. *Statistically significant difference between baseline and endline at $p<0.05$ (McNemar's test). **(A)** Percentage of respondents purchasing AIVs from other farms. **(B)** Percentage of respondents purchasing AIVs from the village market. **(C)** Percentage of respondents purchasing AIVs from the town market.

helped to ensure local food and cultural security. Moreover, the availability of improved germplasm and seed stock for producers could have improved production and yield (42–45). For example, a study by Ojiewo and colleagues (46) found a significant difference in the yielded dry weight, plant height, and flowering time between different varieties of African nightshade. Furthermore, with such rich germplasm, targeted breeding could screen existing germplasm and select for varieties that can withstand climate shocks such as extreme drought. In addition to influencing on-farm availability, climate variability can limit affordability, and accessibility within the larger value-chain.

Across intervention groups, there was a reported shift from sourcing AIVs from village to town market. Smallholder farmers often supply the local village markets and have historically been unable to access the larger commercial value-chain (47). With seasonal drought impeding on-farm production and subsequently decreasing supply to the local village markets, households would be forced to travel to the larger town market, supplied by commercial farmers, to procure the household foods. A study in Kenya by Gido et al. (48) found that retail outlets followed by farm gate outlets were the most preferred food sources in rural households while green groceries were the least preferred. In addition, proximity to household was a major determinate in retail preferences (48). Post-intervention, increased sourcing of food from the town markets, compared to pre-intervention, underscores the limited availability and affordability of AIVs within the local value-chain.

## 4.3. Perception of dietary change post-intervention treatment

Despite a desire to change one's diet, and regardless of intervention, respondents perceived that production remained the greatest influence on AIV consumption post-intervention. The nutrition and culinary interventions were designed in response to community needs using focus group discussions. Moreover, the nutrition and culinary intervention group noted that diet awareness significantly improved diet quality; yet even this intervention group reported production as the single greatest influence on diet quality. AIV production can contribute to household food security and sovereignty, and ecological sustainability through household and community autonomy within the food system (38, 49–52). A study in Petén, Guatemala by Marquez and Schwartz (53) reported that diverse and productive home gardens, contributed to household income, nutrition delivery, and strengthened social bonds and networks. In addition, a review study by Garcia et al. (54) revealed that community cooking classes increased confidence in cooking skills and promoted the consumption of fruits and vegetables.

## 4.4. Limitations

This study relied on recall data and therefore, it is possible that in certain cases the data collected may not be accurate as many

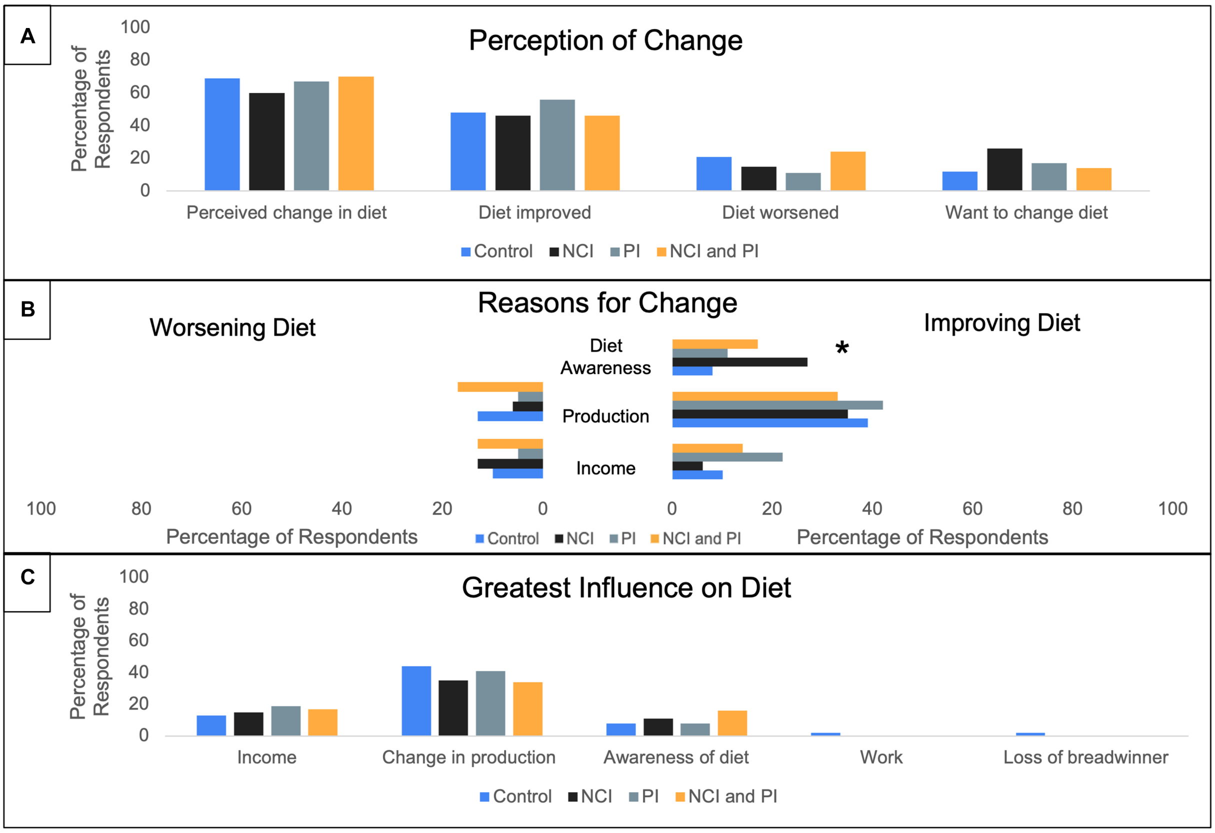


FIGURE 9
Percentage of respondents perceiving dietary changes post-treatment interventions. Multiple answers recorded. *Statistically significant difference between treatments at $p<0.05$ ($\chi^2$ test). **(A)**. Percentage of respondents who reported a perception of dietary change. **(B)** Reported reasons for diet either worsening or improving. **(C)** Reported greatest influences on dietary change.

participants do not keep written records. However, in such cases probing questions were asked to get the most possible accurate data from the respondent. In addition, additional corresponding data collected during a subsequent rainy or dry season for comparison could provide further insights on programmatic impact. Lastly, the use of the full validated FANTA IIII household hunger survey tool could have revealed a more nuanced understanding of diet quality food insecurity in the target communities.

## 5. Conclusion and recommendations

This paper examined the programmatic influence of nutrition, culinary, and production interventions on nutrition security among smallholder farmers Western Kenya. The findings revealed that coupled nutrition, culinary, and production interventions could create a protective effect against seasonal fluctuations in the availability and affordability of AIV as evidenced by a higher Women's Dietary Diversity Score (WDDs). Furthermore, post-intervention, a higher proportion of respondents reported adequate supply of AIVs; however, when they needed to purchase AIVs there was a shift to the town market potentially due to limited availability, accessibility and or affordability at the village market, the preferred retail outlet pre-intervention.

Regardless of treatment, respondents perceived agricultural production to have the greatest influence on diet quality, despite the nutrition and culinary intervention group report that 'diet awareness' had a significant impact on diet quality. Future research directions include evaluating programmatic impact between similar seasons within smallholder farmer groups. Future programming and policy should focus on promoting the availability, accessibility, acceptability, and affordability of improved agronomic practices and germplasm for both smallholder farmers. In addition, agricultural training and extension services should incorporate nutrition and culinary interventions that emphasize the importance of farmers prioritizing harvests for their household consumption. Commercial seed industries, in partnership with regional leaders such as the World Vegetable Center, should prioritize the development improved AIV varieties that contain high levels of micro-and macronutrients, improved agronomic characteristics (e.g., delayed flowering), and are aligned with the communities' cultural preferences.

## 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 Institution Review Board at Rutgers University, the State

University of New Jersey Institutional Research and Ethics Committee at the Academic Model for Providing Access to Healthcare (AMPATH) in Kenya. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## Author contributions

JS, DH, RG, and XM contributed to study design and survey development in concert with MO. NM led the interventions and participated in the field survey including data collection. MO led the survey implementation, enumerator training, and data management. EM analyzed the data and wrote the manuscript draft. 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.2023.1154423/full#supplementary-material

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# Preparation, structure characterization and functional properties of pea dregs resistant dextrin

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**Background:** Peas (*Pisum sativum L.*), the second largest edible bean in the world, have comprehensive and balanced nutrition. In China, peas are mainly used in the processing of starch and related products, during which a large amount of processing by-products—pea dregs—is produced. Because of its large particle size, coarse texture, and difficulty in storage, it is mostly discarded or used as feed, resulting in unnecessary waste.

**Materials and methods:** The preparation and simulated moving bed chromatographic purification process conditions of pea-resistant dextrin were optimized using pea production waste-pea residue as raw material, and structural characterization and functional properties of pea residue-resistant dextrin were analyzed.

**Results:** The results showed that the optimal preparation process conditions for pea-resistant dextrin were as follows: acid concentration 1.0%, acid addition 7.3%, treatment temperature 178.8°C, and treatment time 92.5 min. Subsequently, the pea-resistant dextrin content of 42.15 ± 0.16% was obtained. The optimal SSMB purification conditions were as follows: feed volume 455 g/h, feed volume 682 g/h, circulation volume 346 mL, outlet concentration 24.8 ± 0.2%, purity 99.35 ± 0.17%, and yield 91.08 ± 0.42%. The structural characterization revealed that pea-resistant dextrin had large and variable particle size and amorphous structure; the chemical bond or functional group differences between pea-resistant dextrin and pea starch were not significant; pea-resistant dextrin was a glucose-based dextran with a monosaccharide composition of 2.6% arabinose, 1.5% xylose, and 95.9% glucose, and its molecular weight was (601.1 ± 8.5) × 103 u. Functional characterization revealed that the RS content of pea-resistant dextrin was 92.35%, which had significantly slow digestive properties as well as hypoglycemic and hypolipidemic effects.

**Conclusions:** Using pea dregs to produce pea dregs resistant dextrin has low production cost and significant functional characteristics, which can be widely applied in the food industry.

KEYWORDS

**pea pomace, resistant dextrin, preparation, purification, simulated moving bed chromatography, structural characterization, functional properties**

# 1. Introduction

Peas (*Pisum sativum L.*), the second largest edible bean in the world, have comprehensive and balanced nutrition (Huang et al., 2020). They have a high nutritional value, containing 33.4–47.5% starch, 24.3–30.4% protein, and 14.4–19.5% crude fiber (Gomes et al., 2018). In China, peas are mainly used in the processing of starch and related products, such as vermicelli, during which a large amount of processing by-products—pea dregs—is produced. Because of its large particle size, coarse texture, and difficulty in storage, it is mostly discarded or used as feed, resulting in unnecessary waste (Wu et al., 2020). The starch content of pea dregs is about 20% (Zhou et al., 2016), and if the starch in it can be processed to make a high-quality, high-value-added resistant dextrin, the utilization rate and added value of the peas can be improved significantly.

Resistant dextrin is a short-chain glucose polymer formed by high-temperature cleavage and condensation of starch as a white or pale-yellow powder, a non-sweet, low-calorie indigestible dextrin (Hobden et al., 2015). Resistant dextrin reduces the secretion of digestive tract hormones that regulate the activity of digestive enzymes in the intestine to alter and maintain the balance of the metabolic activity of the body, which promotes human health (Xie et al., 2017). It has the physiological functions of regulating blood sugar (Shigeru et al., 1999; Aliasgharzadeh et al., 2015) and fat metabolism (Basu and Ooraikul, 2010; Dong et al., 2015), improving the composition of human intestinal colonies (Le et al., 2003; Barczynska et al., 2010; Lanubile et al., 2012), and being a laxative (Emilien et al., 2018; Pérez-Calahorra et al., 2020). Also, as a major water-soluble dietary fiber with good processing characteristics, it can be widely used in dairy products, baby food, pasta products, meat products, and other fields and has a broad application prospect. The preparation of resistant dextrin is similar, and the structural characteristics, physiological functions, and other properties of resistant dextrin are related to the raw materials and their starch composition; therefore, the studies on the preparation of resistant dextrin are focused on the selection of raw materials. Shigeru et al. (1991) made resistant dextrin from potato starch. Kapusniak and Jane (2007) prepared maize-resistant dextrin from maize starch. Kamonrat et al. (2019) prepared cassava-resistant dextrin from cassava starch; Liu et al. (2022) prepared mung bean resistant-dextrin from mung bean starch. Zhang et al. (2020) prepared buckwheat resistant-dextrin from sorghum starch. Hitherto, the preparation of pea-resistant dextrin has not been reported. Thus, the present study was carried out to prepare pea dregs-resistant dextrin by acid thermal enzymatic method using pea dregs as raw material. Then, the dextrin was purified by simulated moving bed chromatography, and its physicochemical structure was analyzed by scanning electron microscopy, infrared spectroscopy, X-ray diffraction, gel chromatography-multi-angle laser light scattering method, and gas chromatography-mass spectrometry coupling, and it's *in vitro* digestibility, *in vitro* hypoglycemic effect, *in vitro* lipid-lowering, and other functional properties were also evaluated. Gas scattering and gas chromatography-mass spectrometry analyzed the physical and chemical structure. Therefore, the present study aimed to explore the methods of deep processing of bean starch waste and lay the foundation for the comprehensive utilization and industrialized production of bean residue.

# 2. Materials and methods

## 2.1. Materials

Pea okara was supplied by Shuangta Food Co., L2.2.1td Yantai, Shandong, China. Pea okara was dried at 40°C, crushed, and screened (200 μm).

## 2.2. Methods

### 2.2.1. Preparation of pea resistant dextrin

After impurity removal, the pea dregs were degreased with petroleum ether, extracted with 0.1 mol/L NaOH solution for 3 h, centrifuged at 3,550 × g for 10 min, and the supernatant was used to prepare protein. The precipitate was repeatedly washed with deionized water to remove the yellowish-brown substances in the upper layer of the precipitate until the starch slurry is white, then mixed with 1 mol/L hydrochloric acid to pH 7.0, filtered, dried at 30°C, crushed through 80 mesh sieve, and pea starch was prepared (Kou et al., 2017). Pea starch was dried at 90°C for 1 h and reduce the water content to below 5%, then spray with hydrochloric acid and pyrolysis at 160–200°C for 1–2 h; After completion, taken it out for natural cooling, add water to the pyrolysis product (pyrodextrin) to make a solution, used 1 mol/L NaOH to adjust it to pH 5.5–6.5, 0.5% high temperature resistance α-amylase reacted at 95°C for 1 h, and the 0.4% saccharifying enzyme reacted at 60°C, pH 4.0–4.5 for 2 h, and then concentrated in vacuum to obtain the crude pea resistant dextrin. The resistant dextrin was purified by simulated moving bed chromatography and its structure and function were determined (Li et al., 2020).

### 2.2.2. Optimization of preparation process parameters of pea resistant dextrin

Weighed pea starch 1,000 g, added hydrochloric acid at the concentration of 0.6, 0.8, 1.0, 1.2, and 1.4%, respectively, add hydrochloric acid at the level of 4, 5, 6, 7, 8, 9, and 10% of raw material, pyrolysis temperature at 150, 160, 170, 180, and 190°C, and treatment time at the level of 80, 90, 100, 110, and 120 min, respectively, high temperature resistance α-amylase added was 0.5%, and the reaction was conducted at 95°C for 1 h. The saccharifying enzyme added 0.4%, reacted at 60°C for 2 h, and concentrated in vacuum to obtain crude resistant dextrin. Taking the content of resistant dextrin in pea as an index, the relationship diagram was drawn by using sigma plot to determine the best rotation test center level. On the basis of single factor test, the response surface method was used to optimize the extraction process. The content of pea resistant dextrin was Y, the acid concentration (%) was X1, the amount of acid added (%) was X2, the treatment temperature (°C) was X3, the treatment time (min) was X4, and the test factor level coding in Table 1.

TABLE 1 Factor levels coding table.

| Coding vale | $X_1$ HCl concentration/ % | $X_2$ HCl addition/ % | $X_3$ Temperature/ °C | $X_4$ Time/ min |
|---|---|---|---|---|
| −2 | 0.8 | 6 | 160 | 90 |
| −1 | 0.9 | 6.5 | 165 | 95 |
| 0 | 1 | 7 | 170 | 100 |
| +1 | 1.1 | 7.5 | 175 | 105 |
| +2 | 1.2 | 8 | 180 | 110 |

### 2.2.3. Purification technology of pea resistant dextrin

#### 2.2.3.1. Technology of preparative chromatography

Weighed 99K$^+$310 resin (Dow Chemical Company, Midland, MI, U.S.A.) 200 mL(structural framework: styrene diethylene benzene, functional Group:-$SO_3^-$, homogeneous particle 310 μm), washed it repeatedly with deionized water to remove the damaged resin and impurities in the process, and soak it overnight with deionized water. The self-made preparation chromatography device (10 × 1,200 mm with thermal insulation function, equipped with six-way sample injection valve) was used for the preparation chromatography experiment. The resin was filled into the preparation chromatography column. After filling, the resin was continuously flushed with deionized water until the conductivity of the effluent was consistent with that of deionized water. During this period, the temperature was continuously increased, and the temperature is maintained to 60°C. The resin was ready for use after filling. The preparation chromatographic separation test was carried out with the feed concentration of resistant dextrin of 60%, the feed concentration of 10 mL, the column temperature of 60°C, and the elution flow rate of 1 BV/h. The concentration was determined by the saccharometer, and the purity of the sample was analyzed by HPLC. The single-column elution curve of pea resistant dextrin was drawn according to the test results (Li et al., 2016b).

#### 2.2.3.2. Technology of SSMB chromatography

The SSMB-6Z6L device (National Coarse Chemical Engineering Technology Research Center, Daqing, Heilongjiang, China) was used for the separation of SSMB purified resistant dextrin. The device has 6 chromatographic columns, which are connected in series. Each chromatographic column has to go through three steps of full in and full out (S1), large cycle (S2) and small cycle (S3) in one operation cycle day (Figure 1). Step 1: injected desorber D into the upper end of the first chromatographic column, and release AD (purity) at the lower end, and F (raw material) at the upper end of the third column at the interval, and release BD (resistant dextrin component) at the lower end of the fourth chromatographic column at the interval; Step 2: The material does not enter or exit the system, but only carries out a large cycle; Step 3: injected desorber D into the upper end of the first chromatographic column, and release BD (resistant dextrin component) at the lower end of the fourth chromatographic column in the interval Then switch to the next chromatographic column, carry out three steps in turn, and then cycle until the end of the test (Li et al., 2016a).

### 2.2.4. Structural characterization

#### 2.2.4.1. The molecular weight of pea resistance dextrin

The molecular weight distribution was determined by liquid chromatography using the method of Wu et al. (2010) with slight modifications and a highly efficient size exclusion chromatography (SEC)-18-angle laser light scatter-differential refractive index detector coupled system, (Wyatt Company, Santa Barbara, CA, USA) with a size exclusion chromatography column (TSK G5000 PW 7.5 × 600 mm; Tosoh Company, Tokyo, Japan). The prepared sample (100 μL) was analyzed using ASTRA 6.1 software. The instrument was calibrated with bovine serum albumin, the mobile phase was 0.1 mol/L $NaNO_3$ (containing 0.02% $NaN_3$), the flow rate was 0.4 mL/min, the column temperature was 60°C, and the loading volume was 100 μL (Wu et al., 2010).

#### 2.2.4.2. Monosaccharide composition analysis

Refered to the method of Cao et al. (2021) and make some changes. The whole process of sample treatment requires nitrogen protection, reduction and acetylation, and GC-MS analysis. The chromatographic column is HP-5MS quartz capillary column (30 m × 0.25 mm, 0.25 μm). The monosaccharide composition of pea resistant dextrin was determined by qualitative analysis of monosaccharide according to the peak time of gas spectrum and the ion peak of mass spectrum.

#### 2.2.4.3. Scanning electron microscope measurement

A Ultra-High-Resolution Schottky Scanning Electron Microscope SU7000 (Hitachi High-Technologies, Tokyo, Japan) was used for analyzing and observing morphology of pea resistance dextrin. resistance dextrin granules homogeneously dispersed were fixed with electrically conductive adhesive, coated with gold using an ion sputter coater, observed using SEM, and representative photographs were taken (Wei et al., 2022).

#### 2.2.4.4. Infrared spectrum measurement

The infrared spectrum was measured with a FTIR-1500 Fourier transform infrared spectrometer (Thermo Fisher Scientific, Madison, WI, USA). The sample and KBr were mixed and ground, and the vacuum tablet press was used for tablet pressing, and the infrared spectrum scanning was conducted in the range of 4,000 400 $cm^{-1}$.

#### 2.2.4.5. X-ray diffraction method

The prepared pea starch and purified pea resistant dextrin were dried and crushed, and then measured by Empyrean X-ray diffraction (XRD) (PANalytical B.V. Almelo, Eindhoven, Netherlands). The voltage of the instrument is 40 kV, the current is 40 mA, and the scanning range is diffraction angle (2θ)5∼40°, step is 10.16 steps/s (Sankhon et al., 2013).

### 2.2.5. Other determination methods

Refered to the methods of Li et al. (2015) for the determination of the concentration, purity, yield and resolution of resistant dextrin.

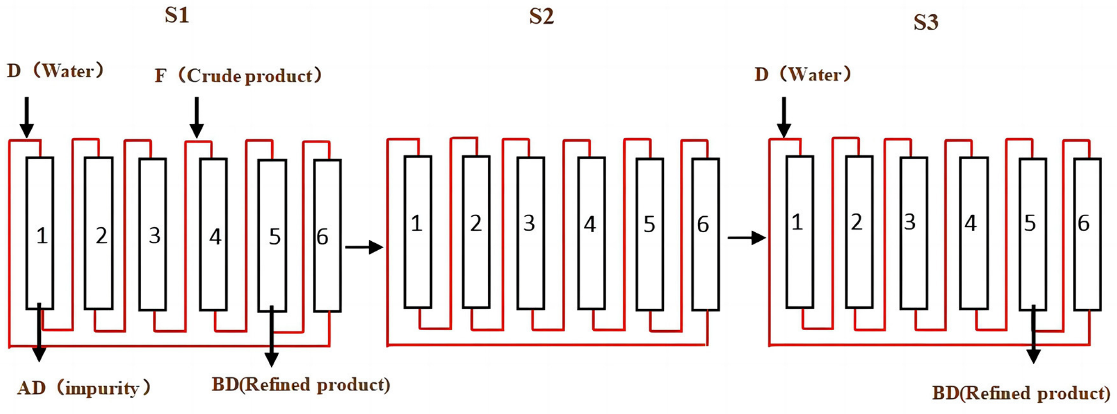


FIGURE 1
The process flow diagram of SSMB.

## 2.2.6. Evaluation of resistant dextrin's functional properties

### 2.2.6.1 Evaluation of *in vitro* simulation of digestion

Refered to the method of Li et al. (2019) and make some changes. Determined the absorbance of the digestive solution at 540 nm, calculate the maltose content in the digestive system according to the standard curve of maltose, and then analyze the content of digestible starch (RDS), slow digestible starch (SDS), and resistant starch (RS) of pea starch and pea resistant dextrin.

### 2.2.6.2. Evaluation of *in vitro* hypoglycemic function

Refered to the method of Ramkumar et al. (2010) and make some changes. Taken acarbose as control, determined the α-inhibition amylase activity and α-inhibition glucosidase activity of resistant dextrin.

### 2.2.6.3. Evaluation of *in vitro* lipid-lowering function

**2.2.6.3.1. Oil-holding capacity**

The oil-holding capacity were determined by reference to Guo et al. (2018) with a slight modifications.

**2.2.6.3.2. Cholesterol adsorption**

The adsorption of cholesterol was determined according to Peerajit et al. (2012) with slight modifications.

## 2.2.7. Statistical analysis

Microsoft Excel (Redmond, WA, USA) and SPSS software (IBM Corp, Armonk, NY, USA) were used for statistical analysis. SigmaPlot (Systat Corporation, Palo Alto, CA, USA) was used for image processing. All data were collected in triplicate and the average value was used for analysis.

# 3. Results

## 3.1. Experimental results and analysis of the preparation of pea-resistant dextrin

The results showed that hydrochloric acid concentration, hydrochloric acid addition, treatment temperature, and treatment time had significant effects on the yield of resistant dextrin ($P \leq 0.05$) (Figure 2). The yield of resistant dextrin in peas increased with the addition of hydrochloric acid; the yield of resistant dextrin was equivalent to the addition of hydrochloric acid >7%, the subsequent increase was not significant, and the yield was maximum with the addition of hydrochloric acid at 7%. Subsequently, the yield of resistant dextrin increased with rising treatment temperature, the increase of yield slowed down and tended to equilibrate with the treatment temperature >170°C, and the yield was maximum with the treatment temperature of 170°C. The yield of resistant dextrin increased with increasing treatment time, and the yield was maximum with the treatment time >100 min. The yield of resistant dextrin increased with increasing treatment time, and the yield of resistant dextrin increased slowly and equilibrated at >100 min. The maximum yield was obtained at 100 min of treatment time. Therefore, a response surface test was conducted with a concentration of 1% hydrochloric acid, 7% hydrochloric acid addition, a treatment temperature of 170°C, and a treatment time of 100 min as the center point.

Based on the results of the single-factor test, a response surface test was conducted using $X_1$ (acid concentration), $X_2$ (amount of acid added), $X_3$ (treatment temperature), and $X_4$ (treatment time) as independent variables and pea-resistant dextrin content as Y. The statistical analysis of the test results showed that the regression model $R^2 = 0.95$, $P < 0.01$, and the $F$-value of the out-of-fit term was 0.21, $P > 0.05$, indicating a good fit for the model. The $F$-value of the primary term was 12.41, $P < 0.01$; the $F$-value of the interaction term was 4.07, $P < 0.05$, and the overall F-value was

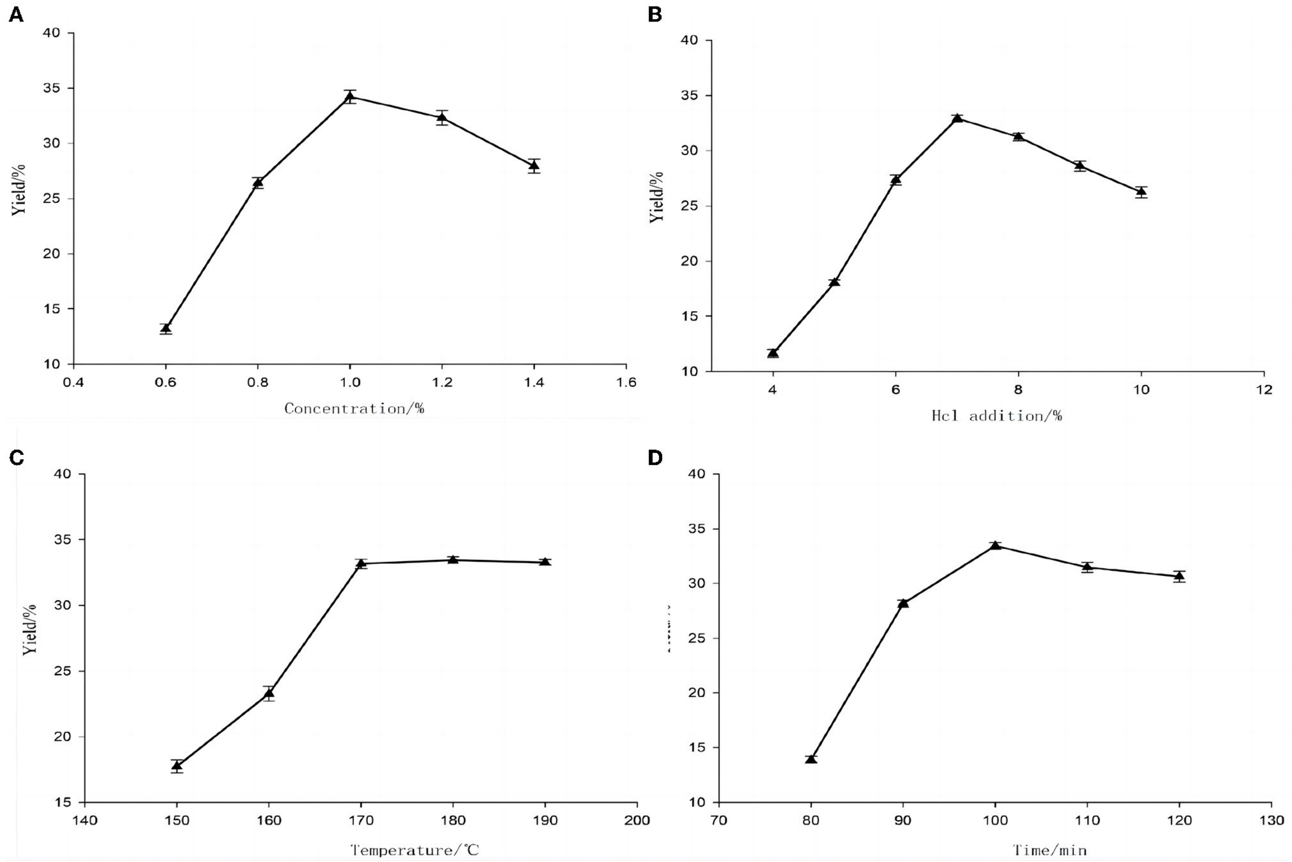


FIGURE 2
Effect of different preparation conditions on the yield of pea resistant dextrin. **(A)** HCl concentration; **(B)** HCl addition; **(C)** Preparation temperature; **(D)** Preparation time.

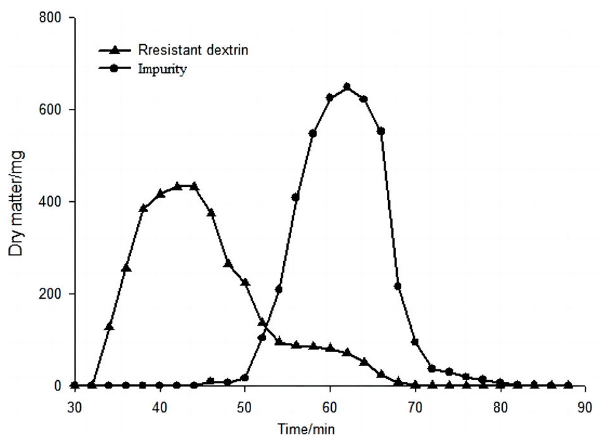


FIGURE 3
The chart of elution curve.

12.39, $P < 0.01$, indicating that each term affected the pea-resistant dextrin content to different degrees. Based on the experimental results, the regression equation $Y = -4559.53 + 641.46X_1 + 99.36X_2 + 32.13X_3 + 22.39X_4 - 481.15X_1^2 - 35.25X_1X_2 + 2.8X_1X_3 + 1.1\,X_1X_4 - 4.7X_2^2 + 0.03X_2X_3 - 0.07X_3^2 - 0.1X_3X_4 - 0.03X_4^2$ was obtained using $X_1$ (acid concentration), $X_2$ (amount of acid added), $X_3$ (treatment temperature), and $X_4$ (treatment time) as independent variables and pea-resistant dextrin content as Y.

Based on the analysis of the regression equation, the standardized values for $X_1$, $X_2$, $X_3$, and $X_4$ were 0.126, 0.302, 0.886, and−0.747, respectively, and the parameters after conversion to non-standardized values were as follows: acid concentration ($X_1$) 1.03%, acid addition ($X_2$) 7.3%, treatment temperature ($X_3$) 178.86°C, treatment time ah ($X_4$) 92.53 min, the theoretical maximum value was 42.38%, and the content of pea-resistant dextrin was 42.15 ± 0.16% obtained from the validation test. The prepared pea-resistant dextrin mainly consisted of five peaks with three main substances: resistant dextrin (peak time 9.974 min), disaccharide (peak time 10.962 min), and glucose (peak time 12.585 min), in which the content of resistant dextrin was 42.1%.

## 3.2. Purification of pea-resistant dextrin

### 3.2.1. Preparation of chromatographic purification techniques

The results of preparative chromatography of pea-resistant dextrin showed (Figure 3) that the retention time of resistant

TABLE 2 Operation conditions and test results on SSMB.

| NO. | Crude product/g·h$^{-1}$ | Water/g·h$^{-1}$ | Circulation volume/mL | Concentration/% | Purity/% | Yield/% |
|---|---|---|---|---|---|---|
| 1 | 455 | 1137.5 | 364 | 11.9 ± 0.2 | 96.55 ± 0.34 | 85.22 ± 0.86 |
| 2 | 455 | 1137.5 | 336 | 10.8 ± 0.3 | 99.39 ± 0.46 | 83.15 ± 0.29 |
| 3 | 455 | 910 | 336 | 16.6 ± 0.4 | 98.12 ± 0.28 | 88.33 ± 0.77 |
| 4 | 455 | 910 | 373 | 18.9 ± 0.2 | 98.79 ± 0.37 | 90.82 ± 0.46 |
| 5 | 455 | 682 | 328 | 22.3 ± 0.3 | 99.32 ± 0.39 | 86.67 ± 0.59 |
| 6 | 455 | 682 | 346 | 24.8 ± 0.2 | 99.35 ± 0.17 | 91.08 ± 0.42 |
| 7 | 455 | 682 | 364 | 25.5 ± 0.3 | 97.21 ± 0.33 | 87.26 ± 0.53 |

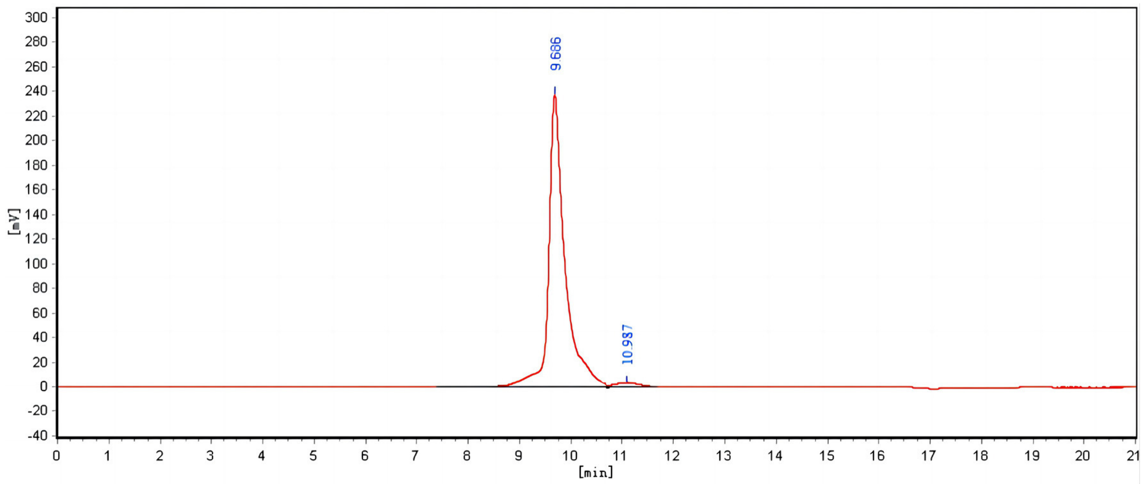


FIGURE 4
Liquid chromatogram spectrum of peas resistance dextrin purified by SSMB.

dextrin and heterosaccharides differed greatly, and the separation was calculated to reach 0.49. Although some overlapping parts could not be separated completely, the separation distance and time could be prolonged by simulated mobile chromatography, the amount of elution feed water could be increased, and a good separation effect could be achieved entirely through the optimization of the experiment.

### 3.2.2. Optimization of process parameters for sequential simulated moving bed purification of pea-resistant dextrin

Based on the results of the preparative chromatographic evaluation tests, the feed refraction of SSMB was determined to be 60%, and the temperature was 60°C. The technical parameters of the purification of resistant dextrin by the SSMB method were optimized (Table 2). Considering the feed volume, feed-water ratio, exit refraction, purity, and yield, the sixth group of tests showed better results than the other six groups. Hence, the best separation conditions were determined as follows: the feed volume was 455 g/h, the feed water volume was 682 g/h, and the circulation volume was 346 mL. At this time point, the exit refraction was 24.8 ± 0.2%, the purity reached 99.35 ± 0.17%, and the yield reached 91.08 ± 0.42%. The liquid chromatogram of the resistant dextrin fraction after separation by SSMB is shown in Figure 4.

## 3.3. Results and analysis of the structural characterization of resistant dextrins

### 3.3.1. Molecular weight determination

The results of molecular weight determination (Figure 5) showed only a single peak on the DPF detection curves of both pea starch and pea-resistant dextrin, indicating that the molecular weights of both molecules were concentrated. The molecular weights (Mw) of pea starch and pea-resistant dextrin were (1465.4 ± 53.2) × $10^3$ u and (601.1 ± 8.5) × $10^3$ u, respectively.

### 3.3.2. Determination of monosaccharide composition of resistant dextrins

The monosaccharide composition of pea starch was more diverse than that of pea-resistant dextrin, containing six monosaccharides, including rhamnose 0.6%, arabinose 6.1%, xylose 4.3%, mannose 1.5%, glucose 82.3%, and galactose 5.2%. Conversely, the monosaccharide composition of resistant dextrin

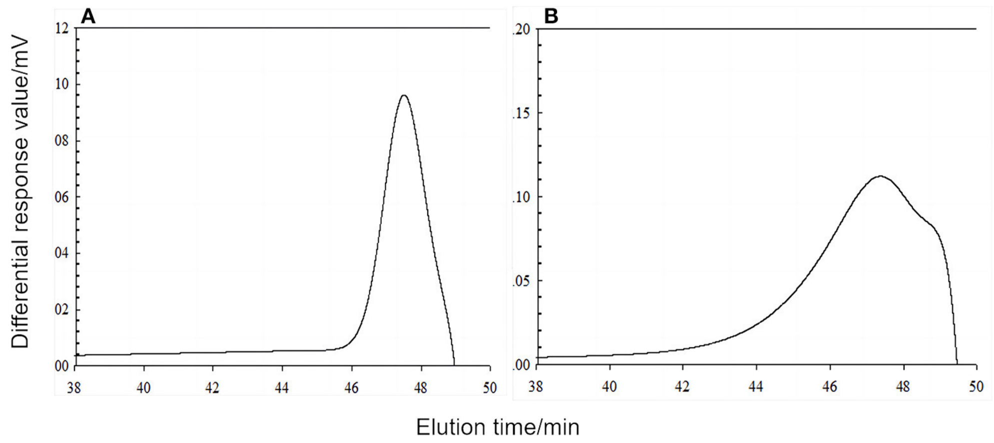


FIGURE 5
Differential refraction detection spectrum of pea starch and peas resistance dextrin. **(A)** Pea starch; **(B)** peas resistance dextrin.

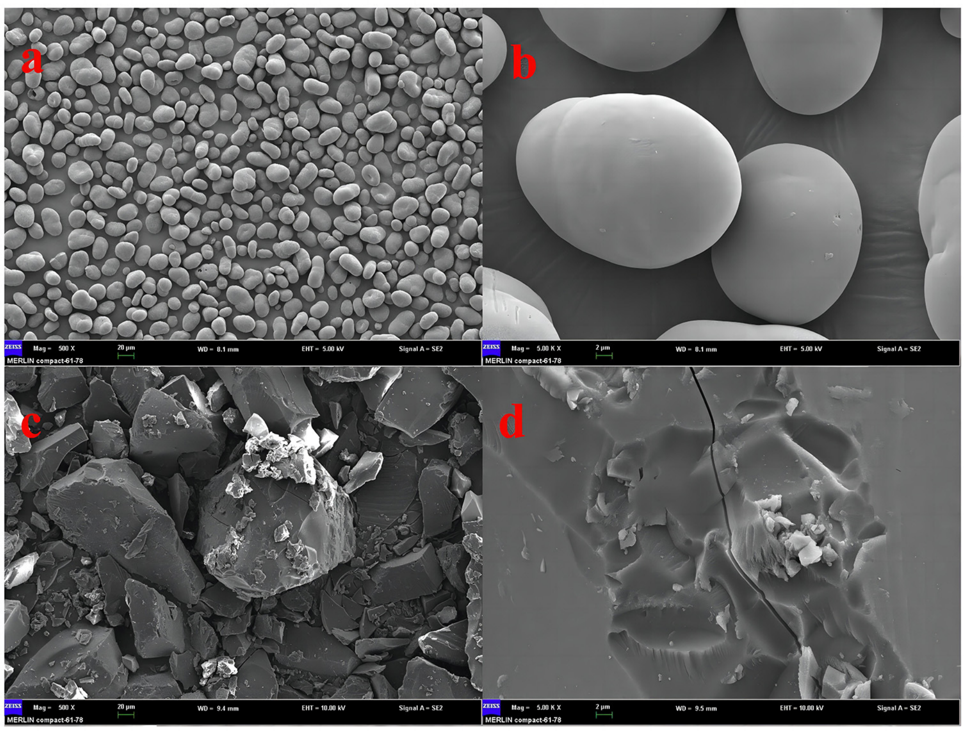


FIGURE 6
SEM images of pea starch and pea resistance dextrin. **(a)** Pea starch (×500); **(b)** pea starch (×5,000); **(c)** pea resistance dextrin (×500); **(d)** pea resistance dextrin (×5,000).

consisted of only three components: glucose as the main ingredient and a small amount of arabinose and xylose. The monosaccharide composition was 2.6% arabinose, 1.5% xylose, and 95.9% glucose. This might be due to the degradation of pea starch under acid heat conditions with glucose as the main body for repolymerization. Also, arabinose and xylose participate in this process, forming a pea-resistant dextrin polymer, while rhamnose, mannose, and galactose may not be involved in and are not detected in the pea-resistant dextrin.

### 3.3.3. Electron microscope determination

The scanning electron microscope results of pea-resistant dextrin showed (Figure 6) that pea starch and purified pea-resistant

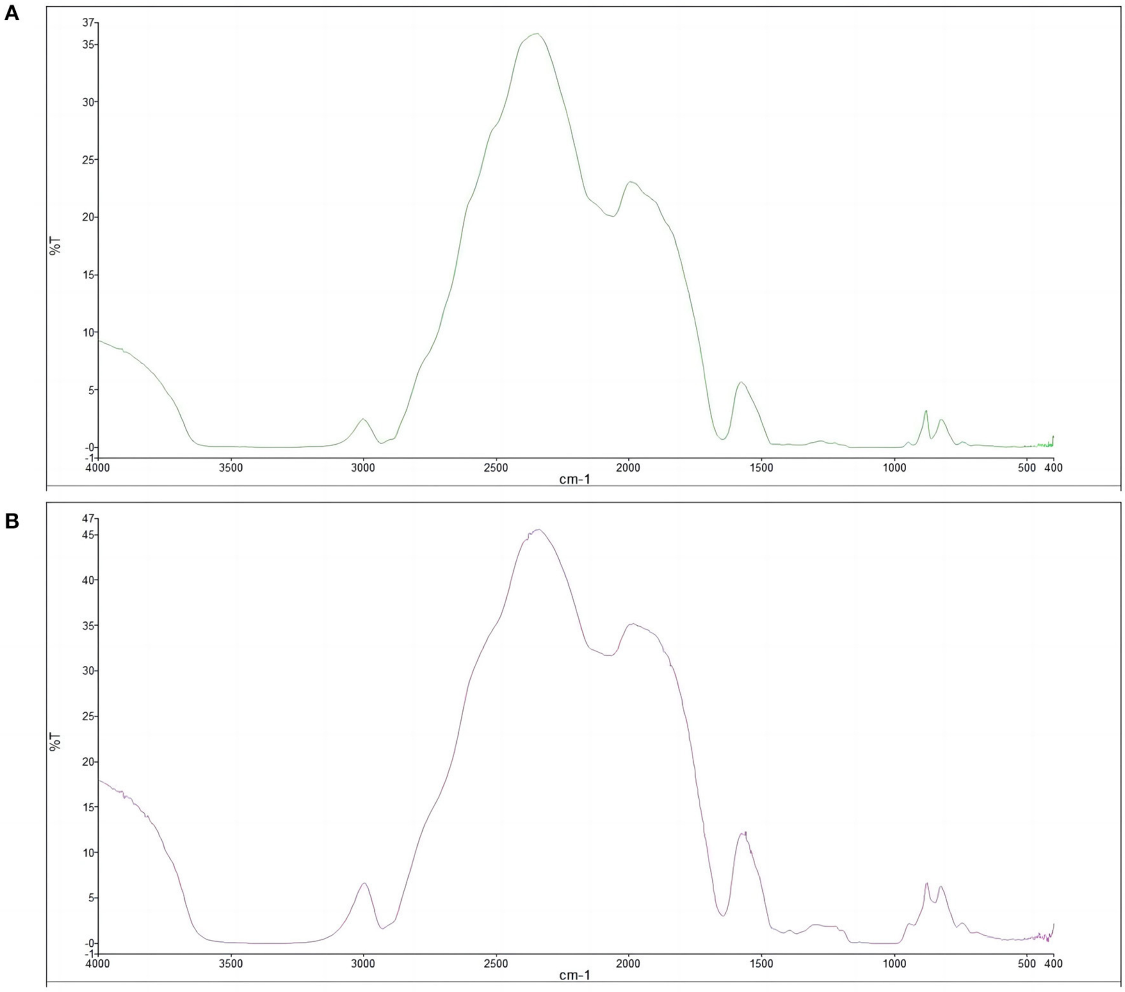


FIGURE 7
Fourier transform infrared spectra of pea starch and peas resistance dextrin. **(A)** Pea starch; **(B)** peas resistance dextrin.

dextrin had completely different morphological structures at the same magnification. The pea starch granules were small, with a slight particle difference, regular oval-shaped granules, and smooth granule surface, while the purified pea-resistant dextrin had larger and different-sized granules and irregular fragments, and the granule surface became rough due to an amorphous structure. This phenomenon could be due to the effect of acid and heat that breaks down the starch molecules, which are reassembled irregularly with different sizes of polymerization. This results in varied sizes and rough surfaces of particles of purified pea-resistant dextrin, favorable for the entry of water molecules, reflecting better solubility and smaller viscosity of pea-resistant dextrin than pea starch.

### 3.3.4. Infrared spectroscopy measurement

The results of the infrared spectra of pea-resistant dextrin (Figure 7) showed that pea starch and pea-resistant dextrin had absorption peaks near 2,900, 2,300, 2,000, and 1,600 $cm^{-1}$. The peak shapes and positions of the characteristic peaks of both infrared spectra did not change significantly, only the size of some absorption peaks differed, which was related to the content of the corresponding chemical bonds. This finding indicated that the chemical bonds or functional groups of pea starch and pea-resistant dextrin had not changed significantly.

### 3.3.5. X-ray diffraction measurement

The results of pea starch X-ray diffraction test showed (Figure 8A) that the crystalline type of pea starch was C-crystalline with 2θ at 15.40, 17.32, and 23.38°, respectively, and the crystallinity was 34.37% by fitting analysis. The X-ray diffractogram of pea-resistant dextrin showed (Figure 8B) that the diffraction peaks of pea-resistant dextrin are broad and not highly crystalline when viewed directly, and its 2θ is about 19°, which is typical of the amorphous spectrum, indicating that pea-resistant dextrin belongs to the amorphous state. This is due to the degradation of pea starch under acid heat conditions, following which the original crystalline structure of pea starch is destroyed; thus, the pea-resistant dextrin is a glucose-based dextran in the amorphous state.

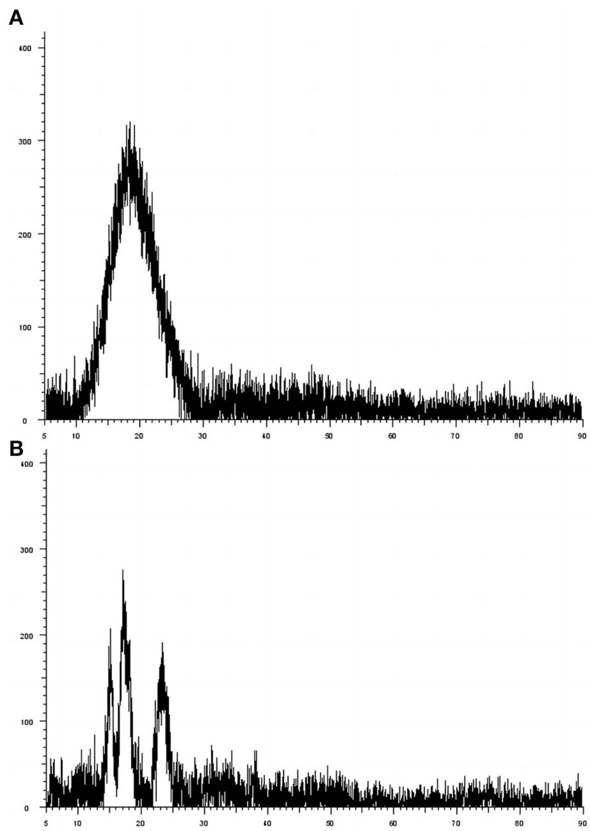


FIGURE 8
The X-ray diffraction spectra of pea starch and peas resistance dextrin. **(A)** Pea starch; **(B)** peas resistance dextrin.

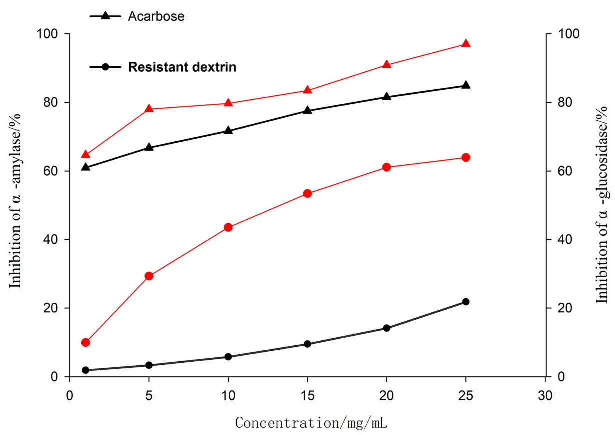


FIGURE 9
The inhibition of α-amylase and α-glucosidase. The black line represents α- Inhibition of amylase activity; The red line represents α- Inhibitory effect of glucosidase.

TABLE 3 Results of cholesterol adsorption by pea resistant dextrin.

| Functional characteristics | | Pea resistant dextrin | Pea starch |
|---|---|---|---|
| Cholesterol adsorption capacity | pH | Adsorption rate (%) | |
| | 7.0 | 28.12 ± 1.58 | – |
| | 2.0 | 22.50 ± 1.16 | – |
| Oil adsorption capacity | Oil type | Adsorption quantity (g/g) | |
| | Lard | 1.78 ± 0.15 | 1.28 ± 0.13 |
| | Soybean oil | 0.88 ± 0.09 | 0.76 ± 0.08 |

## 3.4. Functional properties of resistant dextrins

### 3.4.1. *In vitro* simulation of digestion

The content of RDS, SDS, and RS of pea starch was determined by simulated gastrointestinal fluid digestion: 81.77, 12.36, and 5.87%, respectively. The content of RDS, SDS and RS of pea-resistant dextrin was 5.37, 2.28, and 92.35%, respectively. The starch content of the resistant dextrins was significantly higher ($P < 0.05$) than that of the untreated starch.

### 3.4.2. *In vitro* hypoglycemic function measurement

The *in vitro* hypoglycemic function test results with pea-resistant dextrin (Figure 9) showed that the inhibition rate of α-glucosidase and α-amylase showed a significant increase in pea-resistant dextrin-dependent manner. This phenomenon indicated a positive linear correlation with the inhibition rate of α-glucosidase and α-amylase. The inhibition rates of α-glucosidase and α-amylase of pea-resistant dextrins were significantly lower than the corresponding inhibition capacity of acarbose ($P < 0.05$).

### 3.4.3. *In vitro* lipid-lowering function measurement

The *in vitro* lipid-lowering function of pea-resistant dextrin (Table 3) showed that pea-resistant dextrin had a marked lipid-lowering function. The cholesterol adsorption capacity of pea-resistant dextrin was 28.12 ± 1.58 mg/g at pH 7.0 and 22.50 ± 1.16 mg/g at pH 2.0. The cholesterol adsorption capacity of pea-resistant dextrin at pH 7.0 was significantly greater than that of pea-resistant dextrin at pH 2.0 ($P < 0.05$). The adsorption capacity of pea starch for lard and soybean oil was determined as 1.32 g/g and 0.76 g/g, respectively, the adsorption capacity of pea-resistant dextrin for both was 1.69 and 0.88 g/g, respectively, and the adsorption effect of pea-resistant dextrin for oil and grease was better than that of pea starch ($P < 0.05$).

## 4. Discussion

Currently, the purity of the prepared resistant dextrin is about 40–50%, and purification is essential to reach the international standards of purity >95%. The common purification methods include microbial fermentation, enzymatic methods, and ethanol precipitation. Aboubacar et al. (2006) studied the effect of Brewer's yeast on the purification of resistant dextrin, and the dietary fiber content of purified resistant dextrin reached 90%; however, the microbial fermentation method has several by-products, complex operation, and high production costs, and is not suitable for industrial production. Zhang et al. (2020) purified sorghum-resistant dextrin by enzymatic method and achieved 88.23% purity. Liu et al. (2022) and Su et al. (2022) used the ethanol method for the purification of resistant dextrin, which showed that ethanol precipitation is still the most commonly used and effective method (Liu et al., 2022; Su et al., 2022). The purity of purified resistant dextrin by ethanol precipitation method reaches >95%, but requires several consecutive washes and complicated operation; the ethanol needs to be recycled, the production cost is high, and also serious pollution and safety risks are recorded. The current methods have problems of low product purity, high consumption, and high purification costs. In this study, SSMB chromatography was used for the purification of resistant dextrin, and the purity of the purified resistant dextrin was >99%, and the yield was >90%. This technique uses deionized water as the eluent, with high inlet and outlet concentrations and low production costs, the purity and yield of the product are high, and the industrial mass production can be automated and continuous (Ha et al., 2016).

Structural characterization revealed that the particles of pea-resistant dextrin were large and of different particle sizes and irregular fragments; the surfaces of the particles became rough and different as amorphous structures. The chemical bonds or functional groups of pea-resistant dextrin did not change significantly after acid heat treatment; the monosaccharide composition of pea-resistant dextrin was dominated by glucose and differed significantly ($P < 0.05$) from that of starch monosaccharides. Also, the molecular weight of pea starch pea-resistant dextrin decreased significantly ($P < 0.05$) after acid heat reaction, the glycosidic bonds were reorganized by heat breakage, and the corresponding chemical bond content changed, and the chemical bond content of anti-digestive enzyme hydrolysis increased, resulting in significant changes in physicochemical properties and the molecular weight and monosaccharide composition of resistant dextrin. In addition, the resistant starch content in the resistant dextrin was significantly higher ($P < 0.05$) compared to pea starch-resistant dextrin and exhibited *in vitro* hypoglycemic and hypolipidemic functions, which is consistent with the current findings (Liu et al., 2022; Su et al., 2022). The overall condensation and polymerization processes of resistant dextrin are stochastic, and its cleavage process is fixed. The α-1,4 glycosidic bond of starch is broken by amylase hydrolysis to form small molecules of glucose and disaccharide analogs, which are reconnected and polymerized by α-1,6 glycosidic bond to form resistant dextrin (Li W. et al., 2016). Digestive enzymes and human gastrointestinal fluids can act only on the α-1,4 glycosidic bond and not on this newly formed bond (Kong et al., 2020), leading to a significant decrease in the ratio of fast- and slow-digesting starch in resistant dextrin and a significant increase in the proportion of resistant starch. Moreover, pea-resistant dextrin exhibited inhibitory effects on α-glucosidase and α-amylase. Resistant dextrin is a glucan with a more complex branching structure than starch. Thus, the presence of these irregular structures may provide the resistant dextrin with an effect similar to acarbose in binding to α-glucosidase, inhibiting the activity of α-glucosidase and α-amylase (Jiang et al., 2022).

## 5. Conclusion

The present study was conducted to investigate the preparation, structural characterization, and functional properties of pea residue-resistant dextrin using pea production waste-pea residue as raw material. Based on a single factor, the processing conditions for the preparation of pea-resistant dextrin by acid thermal treatment were optimized by response surface methodology, and pea-resistant dextrin with purity >99% was obtained by preparative chromatography and simulated moving bed chromatography purification. The structural characterization revealed that the resistant dextrins have satisfactory processing properties and can be widely used in the dairy, beverage, noodle products, health food, meat products and other food industry. The functional properties of *in vitro* digestibility, hypoglycemia, and hypolipidemia were analyzed, and we found that pea-resistant dextrin has significantly slow digestibility as well as hypoglycemic and hypolipidemic effects. This study provided novel ideas for the comprehensive utilization of pea dregs, which significantly increased the added value of pea dregs processing and promoted the development of pea cultivation, production, deep processing, and other related industries.

## 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

LL and R-aC were responsible for the design, overall management of the entire study, and writing review and editing. YL, TH, XL, CS, and CL provided the validation and formal analysis. YL, TH, and CL analyzed the data. R-aC supervised, writing—review and editing, and funding acquisition. All authors have read, agreed to the publishing of the current version of the manuscript, 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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# Development of a methodology to compare and evaluate health and sustainability aspects of dietary intake across countries

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To solve the rising issue of how to feed our planet in the future, we need to enhance our knowledge of peoples' current eating patterns and analyze those in terms of their health and environmental impacts. Current studies about adherence to existing national and global dietary recommendations often lack the ability to cross-compare the results among countries. Therefore, this study aims to develop a methodology to evaluate adherence to food-based dietary guidelines (FBDGs) and the Planetary Health Diet (PHD) on a national level, which can be replicable in different countries. First, national dietary intake data was collected from surveys published by the respective responsible public institutions from five countries (Italy, Denmark, Germany, Morocco, and Poland). Second, food groups represented in the intake data and the FBDGs were mapped to establish a proposal for a new common grouping (i.e., comprehensive food groups) that enables cross-country comparison. Third, dietary intake was compared to the recommendations according to national FBDG and the PHD. The adherence to the recommended diets was assessed using an adapted version of the German Food Pyramid Index. Our results show that different ways of grouping foods may change adherence levels; when measuring adherence to the FBDGs with the food groups suggested in the FBDGs, average scores (45.5 ± 5.4) were lower than by using comprehensive food groups (46.9 ± 3.7). Higher adherence to the PHD (52.4 ± 6.1) was found also using the comprehensive food groups. Particularly the foods meats, eggs, and legumes in one group (i.e., protein equivalents) appear to influence the outcome of scores using the comprehensive food groups. This study developed a methodology to evaluate national dietary intake against national FBDGs and the PHD. Our study points out the fact that it is difficult to overcome the challenge that countries have different food grouping clusters. Yet, the combination of the methods developed enables cross-country comparisons and has the potential to be applied to different national settings globally.

KEYWORDS

**SysOrg, food-based dietary guidelines, planetary healthy diet, sustainable diets, adherence to diets, diet quality**

## Introduction

Food-based dietary guidelines (FBDGs) are key tools to promote sustainable healthy diets (Tuomisto, 2018; FAO and WHO, 2019). However, most populations are not fully complying with their recommendations (Leme et al., 2021). Prevailing dietary patterns are unhealthy and unsustainable, requiring a Great Food Transformation to be able to properly nourish people in the future without causing excessive harm to the environment (Willett et al., 2019). This transformation calls for a better understanding of current dietary patterns and analyzing them regarding health and environmental outcomes.

Research has shown that adherence to FBDGs is a good indicator of diet quality regarding both health and environmental impact. Observational studies showed that populations with lower adherence to their FBDGs presented a higher risk for cardiovascular diseases (Ewers et al., 2020) and all-cause mortality (Biesbroek et al., 2017; Ewers et al., 2020). Moreover, previous research has shown that eating accordingly to the FBDGs decreases the dietary environmental impact when compared to current Western dietary patterns (Biesbroek et al., 2017; Arrieta and González, 2018).

In this context, several studies have measured adherence to FBDGs in cohorts using different methods (von Ruesten et al., 2010; Knudsen et al., 2012; Struijk et al., 2014; Biesbroek et al., 2017; Looman et al., 2017; Gómez-Donoso et al., 2019; Ewers et al., 2020). These studies, however, are not easily comparable as most adherence indexes are designed to fit either the dietary assessment tools used, or they evaluate specific aspects of diet (e.g., energy contribution of fats).

Alternatively, dietary patterns can be evaluated against a globally applicable reference diet, such as the Planetary Health Diet (PHD). The PHD comprehends global dietary recommendations proposed by the EAT-Lancet Commission and is designed to have a lower environmental impact and be healthier than current dietary patterns (EAT-Lancet Commission, 2019; Willett et al., 2019). Despite some criticism regarding affordability (Hirvonen et al., 2020), the PHD has been used to evaluate diet quality and sustainability of dietary patterns and guidelines (Sharma et al., 2020; Hendrie et al., 2022). The PHD is also applied as a scientific base for developing dietary guidelines, such as the Danish Dietary Guidelines (Lassen et al., 2020). Moreover, different indexes based on the PHD have been proposed, such as WISH (Trijsburg et al., 2021), and PHDI (Cacau et al., 2021). Despite their strengths, they have not been used to evaluate other populations besides the ones in the original studies. Both FBDGs and the PHD can be important tools to promote sustainable diets, which are diets that "[...] have low environmental pressure and impact; are accessible, affordable, safe and equitable; and are culturally acceptable" (FAO and WHO, 2019).

Considering the potentiality of the FBDGs and the PHD in promoting sustainable healthy diets, it is important to understand to which degree current dietary patterns comply with these references. Therefore, the present study seeks to develop a methodology that evaluates dietary patterns across five nations based on the recommendations of their FBDGs and the PHD on a national level. To our knowledge, this is the first study that elaborates comprehensive food groups to enable a cross-comparison of diet quality across nations.

## Materials

The current study is part of the project "Organic agro-food systems as models for sustainable food systems in Europe and Northern Africa" (SysOrg) (www.uni-kassel.de/go/sysorg, last accessed 13 January 2023) that investigates five case study territories (CSTs) in five countries: Italy, Denmark, Germany, Poland, and Morocco. Therefore, these countries were considered the geographical boundaries of this study.

### Dietary guidelines

For this study, the national FBDGs and complementary materials were used (Table 1). Unlike the European countries, Morocco has no FBDG but a Nutrition Guide (Ministère de la Santè, 2016) intended to be used by healthcare professionals, which for this study was used equivalently as a substitute. The recommendations from Italy, Morocco, and Poland were available only in the local languages and were translated using Google Translator (Italian: Sep/2021; French and Polish: Jan/2022). The complementary materials were used to assess the portion sizes established for each population and are listed in Table 1.

Recommended daily dietary intakes were established by applying upper values as reference (e.g., the Moroccan Nutrition Guide recommends two servings a day of vegetables ranging from 150 to 300 g each, so 300 g was established as the portion size). Moreover, the recommendations were adjusted for daily intake. Therefore, weekly recommendations were divided by seven (e.g., Italian FBDG recommends an upper intake of 100 g of red meats a week, so daily recommendations are 100/7 = 14.28 g/day).

### Dietary intake

Average intake information was extracted from national dietary intake surveys and reports (Table 2). Only mean values were considered for this step, and data were converted to daily intake in grams or milliliters when needed. When possible, dietary intake collected was from adults, since FBDGs are calculated for adults with a 2,000 kcal or 10 MJ energy requirement (Oberritter et al., 2013; Danish Veterinarian and Food Administration, 2021). Data on alcohol intake was not available in all countries. Considering the impact of alcoholic beverages on diet and health (Johnson et al., 2022), data on alcohol intake was extracted from the Global Health Observatory (WHO, 2019) for the five countries. Values considered for calculating adherence scores are available in the Supplementary material.

### The Planetary Health Diet

Reference values from the PHD were extracted from Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems (Willett et al., 2019) considering the midpoint values proposed in the reference diet. Reference values for legumes were given for dry pulses, but for this study, we used 175 g

TABLE 1 List of materials used for recommended intake of food groups.

| Country | FBDG* | Complementary material** |
|---|---|---|
| Denmark | The official dietary guidelines-good for health and climate[a] | Development of a Danish adapted healthy plant-based diet based on the EAT-lancet reference diet[b] |
| Germany | Ten guidelines of the German Nutrition Society for a wholesome diet[c] | The DGE Nutrition Circle–Presentation and Basis of the Food-Related Recommendations from the German Nutrition Society (DGE)[d] |
| Italy | Dietary guidelines for healthy eating– revision 2018[e] | - |
| Morocco | Moroccan Nutrition Guide for use by healthcare professionals[f] | Dietary and health guidelines for the preparation of menus at the level of university residences and boarding schools in higher education establishments[g] |
| Poland | Healthy eating recommendations: plate of healthy eating[h] | Check how many servings of different products you can eat during the day[i] |

*Assessed in September 2021. **Assessed in January and February 2022. [a]Danish Veterinarian and Food Administration (2021). [b]Lassen et al. (2020). [c]The German Nutrition Society (DGE) (2017). [d]Oberritter et al. (2013). [e]CREA (2019). [f]Ministère de la Santè (2016). [g]Ministère de la Santè (2013). [h]Narodowe Centrum Edukacji Zywieniowej (NCEZ) (2020b). [i]Narodowe Centrum Edukacji Zywieniowej (NCEZ) (2020a). Adapted with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

TABLE 2 Dietary intake surveys and their methodology, time frame and data extracted.

| Country | Survey | Methodology | Population and data extracted |
|---|---|---|---|
| Denmark | Dietary Habits in Denmark 2011–2013[a] For a healthier and more sustainable diet [b] | 7 consecutive days pre-coded food record questionnaire | 3,016 adults (18 to 75 years), 1,552 females Mean individual daily intake, in total and per sex |
| Germany | Results of the National Nutrition Monitoring, survey year 2014[c] | Food consumption and dietary patterns were assessed by dietary history interviews and 24-h recalls | 1,508 adults (22 to 80 years), 868 females Mean individual daily intake, per sex and age groups (22–50 and 51–80 years) |
| Italy | The Italian National Food Consumption Survey INRAN-SCAI 2005–06[d] | A cross-sectional study performed with randomly selected households; food consumption was assessed on three consecutive days through individual estimated dietary records | 2,312 adults (18 to 64.9 years), 1,244 females Mean of individual daily consumption (3 days average), in total and per sex |
| Morocco | The National Survey on Household Consumption and Expenditure 2013/2014[e] | Food consumption per household was assessed by frequency of food purchase | 15,970 households Average annual intake per capita |
| Poland | Household budget survey in 2019[f] | Food consumption was measured by assessing food purchased, received for free and taken activity | 35,923 households', 93759.03 participants (average number of people living in a household: 2.61) Average monthly consumption per capita |

[a]Pedersen et al. (2015). [b]Trolle et al. (2019). [c]Bundesministerium für Ernährung und Landwirtschaft Max Rubner Institute (2014). [d]Leclercq et al. (2009). [e]Haut Commissariat au Plan (2016). [f]GUS (2019). Adapted with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

of cooked legumes for the score, considering a 2.5 weight change factor, as proposed in the work of Lassen et al. (2020).

## Methods

In this study, three adherence scores were developed for different purposes (Table 3). The first score (ADH1) measures adherence to FBDGs using the corresponding food groups established by each country, resulting in a tailored score for each population. For comparison purposes, a second score (ADH2) was designed to compare adherence levels to the FBDGs across different populations. The third score, (APHD) was designed to assess adherence to the PHD, allowing a comparison of the different populations using a common diet as a reference.

### Methods development steps

#### Step 1-identifying the food groups

Step 1 consisted of identifying the food groups represented in the materials and assuring that all food groups were represented in the recommendations and intake sources (Tables 1, 2). For better visualization, a color scheme was created for the food group mapping, as seen in Table 4.

The FBDGs of the five CSTs show different food groups (Table 4). For the ADH2 and APHD, this difference required the creation of a new common food group distribution to allow cross-comparison between different populations. The new classification generated seven comprehensive food groups, following groupings suggested by the FBDGs as displayed in Table 5.

All food groups and comprehensive food groups were classified into "positive," "neutral," and "negative" for health, as seen in Table 5. The classifications are based on scientific evidence, following assortments proposed by other authors (von Ruesten et al., 2010; Gómez-Donoso et al., 2019).

#### Step 2-upper intake of alcohol and sweets

Table 4 displays that none of the FBDGs have recommended intake for the groups in the "negative" class. Therefore, following similar studies (Gómez-Donoso et al., 2019), a value of upper-limit consumption was determined for the negative food groups. The determined values were:

- Sugar and sweets (Ygil, 2013):

TABLE 3 Adherence scores developed for food-based dietary guidelines and the Planetary Health Diet.

| | ADH1 | ADH2 | APHD |
|---|---|---|---|
| Name | Adherence to FBDG | Adherence to FBDG using comprehensive food groups | Adherence to the PHD |
| Index dimensions | Health impact | | Health and environmental impact |
| References | FBDG recommendations and national dietary intake | FBDG recommendations and national dietary intake, in comprehensive food groups | PHD recommendations and national dietary intakes, in comprehensive food groups |
| Scores per food group | Class 1 (positive): 0 to 10; up to 10 extra points could be given for extra intake<br>Class 2 (neutral): 0 to 10; extra intake caused proportional deduction of points<br>Class 3 (negative): 0 to 10; calculated inversely (10 is no intake) | | |
| Maximum total score | IT: 190 (150 + 40 extra points)<br>DK: 180 (150 + 30 extra points)<br>DE: 130 (110 + 20 extra points)<br>MA: 140 (110 + 30 extra points)<br>PL: 110 (90 + 20 extra points) | For the five countries: 80 (70 for the seven food groups + 10 extra points from the positive class) | |
| **Methods development steps** | | | |
| Step 1 | Identification and color coding the identified food groups present (Table 4) | | |
| | | Establishment of comprehensive food groups for comparing countries (Table 5) | |
| Step 2 | Establishment of upper limit intake for alcohol and sweets [Ygil, 2013; Gómez-Donoso et al., 2019; National Institute on Alcohol Abuse and Alcoholism (NIAAA), n.d.] | | |
| Step 3 | Assessment of health impacts of the food groups and classification of them accordingly | | |
| Step 4 | Calculation of score for each food group using Eq1 and Eq2 according to groups classifications (von Ruesten et al., 2010) | | |

FBDG, Food-Based Dietary Guidelines; PHD, The Planetary Health Diet (Willett et al., 2019); IT, Italy; DK, Denmark; DE, Germany; MA, Morocco; PL, Poland. Adapted with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

- One serving of 25 g/d

- Alcohol [Gómez-Donoso et al., 2019; National Institute on Alcohol Abuse and Alcoholism (NIAAA), n.d.]:
  - For females: 1 dose/d or 14 g in pure alcohol/d
  - For males: 2 doses/d or 28 g in pure alcohol/d
  - For the general population (males and females): 1.5 doses/d or 21 g in pure alcohol/d

## Step 3-health impacts of the food group

The first class, "positive," has food groups with protective effects for diet-related diseases and is composed of fruits, vegetables, potatoes, and water or unsweetened drinks (for Italy only). The "neutral" class consisted of food groups whose intake should be within recommendations, as their excessive intake could be harmful to health, consisting of cereals, dairy, protein equivalents, and fats. The third class, "negative," consists of food groups that the FBDGs recommend limiting the consumption or eating in moderation, which in this study were sugar and sweets, and alcohol. These classifications were later (i.e., step 4) used as a reference for scoring the intake of each food group.

## Step 4-calculation of score for each food group

The three adherence scores (ADH1, ADH2, and APHD) were developed inspired by the German Food Pyramid Index (GFPI) (von Ruesten et al., 2010) but adapted from servings/day to gram/day. Each class of food groups was scored differently. The score for food groups within the "positive" class was calculated using the following equation:

$$\text{food group score} = \frac{\text{average consumption/d}}{\text{recommended intake/d}} \times 10 \quad (1)$$

Considering the potential health benefits of higher consumption than recommended, up to 10 extra points could be given for extra intakes, following Eq1.

The scores for "neutral" food groups were also obtained with Eq1. However, intake of foods surpassing the recommendations in this class was deduced. The deduction of points was done by calculating their score using Eq2, as follows:

$$\text{food group score} = \frac{\text{recommended intake/d}}{\text{average consumption/d}} \times 10 \quad (2)$$

The "negative" food groups' scores were calculated by using Eq2, which means 10 points were given for consumption below the upper limit. In case of no intake, 10 points were given.

Scores were calculated using Microsoft® Excel version 16.59. Adherence to FBDGs (ADH1) was measured differently in each country, as it considers each country's particularities. Italian national adherence was done for adult males and females separately, and they could be scored from 0 to 200 (160 points from the 16 food groups + 40 possible extra points in the four positive groups). The Danish population was assessed using the intake of the general adult population, and separately for adult males and females, being scored from 0 to 170 (140 points from the 14 food groups + 30 possible extra points). Adherence to the German FBDG was assessed for adult men and women separately and scored from 0 to 130 (110 points for 11 food groups + 20 possible extra points).

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**TABLE 4** Identification of food groups represented in the recommendations and in the dietary intake surveys per country for Italy, Denmark, Germany, Morocco, and Poland.

| Italy | | Denmark | | Germany | | Morocco | | Poland | |
|---|---|---|---|---|---|---|---|---|---|
| Recommendation | Intake | Recommendation | Intake | Recommendation | Intake | Recommendation | Intake | Recommendation | Intake |
| Vegetables | Vegetables | Vegetables | Vegetables | Vegetables | Vegetables | Vegetables | Vegetables | Vegetables | Vegetables |
| Fruits | Fruits | Fruits | Fruits | Fruits | Fruits | Fruits | Fruits | Fruits | Fruits |
| Red meats | Red | Red meat | Red meat | Meats | Meats | Red meats | Meats | Meats | Red meat |
| White meat | White | Poultry | Poultry | | | White meat | | | Poultry |
| Fish | Fish | Fish | Fish | Fish | Fish | Fish | Fish | | Fish |
| Eggs | Egg | Eggs | Eggs | Eggs | | Eggs | Eggs | | Eggs |
| Legumes | Legumes | Legumes | Legumes | | | Legumes | Legumes | | Legumes |
| Dairy | Dairy | Dairy | Dairy | Dairy | Dairy | Dairy | Dairy | Dairy | Milk |
| Cheese | Cheese | Cheese | Cheese | Cheese | Cheese | | | | Cheese |
| Oils and fats | Oils and fats | Oils and fats | Oils and fats | Oils and fats | Oils and fats | Oils and fats | Oils and fats | Oils and fats | Oils and fats |
| Cereals | Cereals | Cereals | Cereals | Cereals | Cereals | Cereals | Cereals | Cereals | Cereals |
| Potatoes | Potatoes | Potatoes | Potatoes | Potatoes | Potatoes | Potatoes | | Potatoes | Potatoes |
| | Alcohol | | Alcohol | | | | | | |
| | Sugar and sweets | | Sugar and sweets | | Sugar and sweets | | Sugar and sweets | | Sugar and sweets |
| Nuts and oily seeds | Nuts | | | | | | | | |
| Water | Water | | | | | | | | |

Foods in the same colors are in the same food group. Reprint with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

TABLE 5 Comprehensive food groups, their respective classification and food groups they contain.

| Food group class | Comprehensive food group | Food groups present |
| --- | --- | --- |
| Positive | FVP | Fruits<br>Vegetables<br>Potatoes |
| Neutral | Cereals | Cereals<br>Grains |
| Neutral | Dairy | Dairy<br>Milk and yogurt<br>Cheese |
| Neutral | Protein equivalents | Red meat<br>White meat<br>Meats<br>Eggs<br>Fish<br>Legumes |
| Neutral | Fats | Fats |
| Negative | Sweets | Sweets |
| Negative | Alcohol | Alcohol |

Reprint from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022). Food group classification are based on the work of von Ruesten et al. (2010) and Gómez-Donoso et al. (2019).

In Morocco and Poland, dietary intake data sources were household surveys with an estimated average intake per capita (Table 2), therefore the analysis is based on the general populations (males and females of all ages) of those countries. The Moroccan population intake was scored from 0 to 140 (120 points for 12 food groups + 20 possible extra points), while the Polish population could be scored from 0 to 110 (90 for 9 food groups + 20 possible extra points).

# Results

The results present the scores calculated for the three different adherence scores (ADH1, ADH2, and APHD), followed by an overview of the scores' results observed, unraveled by the food groups. Lastly, we present the data from the dietary intake surveys collected for comparison. This study focuses on the description of the methodology developed.

## The comprehensive food groups

Cross-comparison of diet quality between the populations in Denmark, Germany, Italy, Morocco, and Poland was only possible due to the proposal of a common food grouping, the comprehensive food groups (Table 5). Compared to the food groups proposed by most of the FBDGs (Table 4), the main differences in the comprehensive food groups were "FVP" and "protein equivalents." Fruits and vegetables were grouped mainly because in Morocco their recommendations are given together without specifying how to divide the recommendation between both groups. Similarly, in Poland, potatoes are included in the recommendations for vegetables as interchangeable, while in other countries potatoes intake has been presented separately.

As for "protein equivalents," Denmark and Italy were the only two countries to have all meats, eggs, and legumes separately. For Morocco and Germany, data was presented with fish separated from other meats and eggs. Due to the Polish recommendations, all protein sources besides dairy were grouped together, without specifying a frequency intake for specific foods, that is, all foods in the "protein equivalents" are presented as interchangeable.

## The three different scores

The three different scores applied to the studied populations showed different scores as seen in Figure 1, using a maximum total score of 80 for the comparison. Regarding adherence to FBDG, all countries had higher scores when evaluated with the comprehensive food groups (ADH2). For all countries except Poland, adherence scores were higher for the APHD than for the methods comparing adherence to national recommendations (ADH1 and ADH2). For Poland, similar scores for different methodologies were observed for ADH2 and APHD. The average total scores for the indexes were 45.5 (±5.4) for ADH1, 46.9 (±3.7) for ADH2, and 52.4 (±6.1) for APHD.

## Adherence to food-based dietary guidelines (ADH1 and ADH2)

The ADH1 score assesses the degree to which the actual consumption of the population, according to national intake data, complies with the national FBDGs. Table 6 presents an overview of the scores given to each population per food group and the overall score. In the countries where red meats were evaluated separately from other meats (i.e., Denmark and Italy) the populations showed an intake of red meats that surpassed the recommendations. In Italy and Denmark, the intake of other animal proteins (i.e., dairy and eggs) was also higher than suggested by their FBDGs.

For cross-comparison purposes, the ADH2 used the same food groups (i.e., the comprehensive food groups), for which the results are displayed in Table 7. For ADH2, Danish females had the highest score, which means they adhere the best to the recommendations while the lowest adherence was observed in German females. In this scoring system, Danes and Italians were scored inversely for protein equivalents for having intakes surpassing the recommendations. For dairy, higher intakes than recommendations were observed in Danish adults.

## Adherence to the Planetary Health Diet

Italian females had the closest diet to the PHD recommendations, as indicated by the highest APHD score (Table 8). Contrasting, both German females and German males had diets that differ the most from the PHD. In the APHD, the cereals food group was often scored inversely, with most populations surpassing the recommended intake.

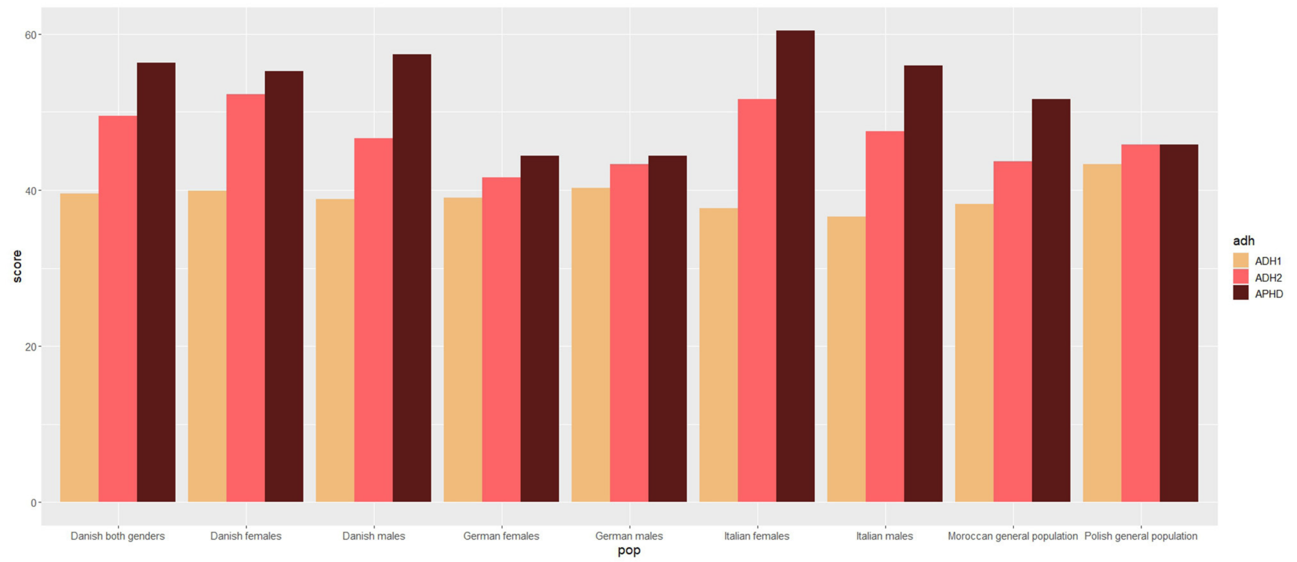


FIGURE 1
Proportional ADH1 total scores and ADH2 and APHD total scores. Total scores in y-axis and studied populations in x-axis. Adapted from *Diet quality and sustainability in different countries and territories* (Philippi Rosane, 2022).

## Discussion

This study proposed a methodology with three different indexes for evaluating diet quality considering health and environmental impacts. Additionally, it displays how some of the difficulties to compare diet quality across nations can be met but still includes limitations due to eating cultures and that food groups differ significantly across nations and intake surveys differ in methods.

The starting point was the development of dietary quality indexes that considered the particularities of five different populations in Italy, Denmark, Germany, Morocco, and Poland. Considering the environmental burden of current food systems, it is imperative that sustainability is considered when evaluating diets (FAO and WHO, 2019). Moreover, comparing diet quality between different populations (i.e., cross-country comparison) can construct a better understanding of the challenges people face to adhere to sustainable healthy diets.

Several diet quality indexes have been developed and validated in previous studies (Waijers et al., 2007), but they cannot be tailored to different populations, and most do not consider the environmental impacts of diets. For this study, the FBDGs were chosen as references since they are tailored to the nutritional needs and dietary habits of the country where they were developed (FAO, 2007). Other studies have investigated adherence to FBDGs but lacked the environmental impact perspective (von Ruesten et al., 2010; Knudsen et al., 2012; Struijk et al., 2014; Biesbroek et al., 2017; Looman et al., 2017; Gómez-Donoso et al., 2019; Ewers et al., 2020). In this study, we proposed as a solution the adherence to the PHD as a complementary measurement of diet quality, being a reference of a sustainable healthy diet.

Recent quality indexes based on the PHD tried to assess the sustainability of dietary patterns in cohort studies, such as PHDI (Cacau et al., 2021), and WISH (Trijsburg et al., 2021). These indexes can be globally applicable but lack to consider the nutritional particularities of each country. Another index that considers health and environmental outcomes is SHED (Tepper et al., 2021), however, it requires that subjects answer a questionnaire about their dietary habits, which is not always possible. In this study, dietary intake surveys were used, which are often publicly available.

### Use of comprehensive food groups

The use of comprehensive food groups was proposed as a way of enabling a cross-country comparison of adherence to reference diets per food group. The results show that the use of comprehensive food groups increased the total scores of all populations. However, the discrepancies in the total scores (Figure 1) for ADH1 and ADH2 suggest that adherence scores with comprehensive food groups should not be used by themselves as they might dilute excessive and very low intakes of certain food groups. In ADH1, the intake of red meats was assessed separately from other meats for countries where there were specific recommendations for red meats (i.e., Denmark, Germany, and Italy). In these three countries, the intake of red meats was higher than national recommendations, having their adherence score calculated inversely to deduce points from excessive intake (Table 6). In ADH2, excessive intake is no longer observed as these populations eat less fish and legumes than recommended. Therefore, we see a dilution of the low scores that would occur for excessive intake and for not meeting the recommendations, which benefitted the total scores of Danes, Germans, and Italians.

Similarly, the higher scores observed for APHD can properly be explained by the use of comprehensive food groups due

TABLE 6 Populations' scores for adherence to the FBDGs, using the ADH1 method, by food group and total score.

| Population | FVP | Cereals | Dairy | Protein equivalents | Fats | Sweets | Alcohol | Total score/maximum score |
|---|---|---|---|---|---|---|---|---|
| Danish both genders | Fruits: 6.3<br>Vegetables: 6.6<br>Potatoes: 9.1 | 5.6 | Milk and yogurt: 8.2*<br>Cheese: 4.5* | Red meats: 1.1*<br>White meats: 8.7<br>Fish: 7.4<br>Eggs: 6.3*<br>Legumes: 0.2 | 7.1* | 6.8 | 6.1 | 83.9/170 |
| Danish females | Fruits: 5.5<br>Vegetables: 6.9<br>Potatoes: 6.5 | 4.8 | Milk and yogurt: 9.2*<br>Cheese: 4.9 * | Red meats: 1.5*<br>White meats: 8.0<br>Fish: 6.8<br>Eggs: 6.5*<br>Legumes: 0.2 | 8.3* | 7.1 | 8.5 | 84.8/170 |
| Danish males | Fruits: 7.1<br>Vegetables: 6.4<br>Potatoes: 8.5* | 6.4 | Milk and yogurt: 7.4*<br>Cheese: 4.3 * | Red meats: 0.9*<br>White meats: 9.7<br>Fish: 8.0<br>Eggs: 5.8*<br>Legumes: 0.2 | 6.8* | 6.6 | 5.2 | 82.5/170 |
| German females | Fruits: 6.3<br>Vegetables: 3.5<br>Potatoes: 2.5 | 8.0 | Milk: 5.4<br>Cheese: 8.2 | Meats: 8.3<br>Fish: 5.1 | 4.7 | 4.8 | 6.6 | 63.4/130 |
| German males | Fruits: 5.3<br>Vegetables: 3.4<br>Potatoes: 3.0 | 9.9 | Milk: 6.2<br>Cheese: 8.2 | Meats: 7.0<br>Fish: 7.3 | 6.7 | 4.3 | 4.1 | 65.6/130 |
| Italian females | Fruits: 4.8<br>Vegetables: 4.3<br>Potatoes: 8.1 | 7.6 | Milk and yogurt: 3.7<br>Cheese: 7.9* | Red meats: 1.8*<br>White meats: 4.3<br>Fish: 8.9<br>Eggs: 8.7<br>Legumes: 1.7 | 7.7* | 8.1 | 10** | 94.1/200 |
| Italian males | Fruits: 4.4<br>Vegetables: 4.6<br>Potatoes: 9.5 | 9.7 | Milk and yogurt: 2.9<br>Cheese: 6.5* | Red meats: 1.3*<br>White meats: 5.3<br>Fish: 9.7<br>Eggs: 8.8*<br>Legumes: 1.8 | 6.5* | 6.8 | 6.4 | 91.5/200 |
| Moroccan general population | Fruits: 3.1<br>Vegetables: 5.7 | 7.3 | 2.4 | Meats: 5.2<br>Fish: 4.3<br>Legumes: 11.1 | 9.8* | Sugar: 3.7 | 10.0** | 66.8/140 |
| Polish general population | Fruits: 7.9<br>Vegetables: 5.1<br>Potatoes: 7.6* | 8.9 | 2.6 | Meats and protein equivalents: 9.8 | 8.9 | Sugar and sweets: 4.6 | 5.2 | 59.5/110 |

*Score by equation 2, because intake surpasses the recommendations.

**Score of 10 was given for intakes of negative food groups that were lower than the upper levels. FVP, fruits, vegetables, and potatoes. Reprint with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

to the grouping of all meats and legumes in the group of "protein equivalents." The PHD advocates a plant-based diet, with low or no intake of meats (Willett et al., 2019). Legumes are proposed as healthy and sustainable protein sources and as alternatives to meats (Fabricius et al., 2021). The PHD suggests a daily intake of 178 g of pulses in a 10 MJ diet (Lassen et al., 2020), which is a goal far from the consumption of the studied populations; European countries in our study have a habitual intake below 12 g/d (Supplementary material). In contrast, in Morocco, the consumption of legumes is substantially higher than in Europe (Supplementary material). At the same time, the Moroccan Nutrition Guide is the only guideline, among the five analyzed in this study, recommending the daily consumption of meat (Supplementary material; Ministère de la Santè, 2016), which could implicate in an increased intake of meats.

As previously mentioned, the "protein equivalents" group was created because in Poland the dietary recommendations suggest one daily portion of either meat (beef, chicken, fish, other), eggs, or legumes [Narodowe Centrum Edukacji Zywieniowej (NCEZ), 2020a], without specifying a minimum or maximum for each food. The Polish FBDG [Narodowe Centrum Edukacji Zywieniowej (NCEZ), 2020a], however, advocates increasing the intake of legumes and fish and decreasing the intake of red and processed meats. Additionally, the Polish FBDG recommends replacing meat with plant-based protein, fish, and eggs. Despite grouping animal and plant-based proteins together, the consumption of those foods is not balanced. According to the Household Budget Survey from 2019 (GUS, 2019), monthly per capita intake of meats is on average 5.08 kg, while consumption of fish is 0.27 kg/month and consumption of legumes is not reported, which might indicate that intake of legumes is low.

TABLE 7 Adherence scores to FBDG according to comprehensive food groups (ADH2).

| Population | FVP | Cereals | Dairy | Protein equivalents | Fats | Sweets | Alcohol | Total score |
|---|---|---|---|---|---|---|---|---|
| Danish both genders | 6.9 | 5.6 | 7.8* | 9.4* | 7.1* | 6.8 | 6.1 | 49.5 |
| Danish females | 6.2 | 4.8 | 8.6* | 8.7 | 8.3* | 7.1 | 8.5 | 52.3 |
| Danish males | 7.4 | 6.4 | 7.0* | 7.8* | 6.2* | 6.6 | 5.2 | 46.7 |
| German females | 4.0 | 8.0 | 5.9 | 7.6 | 4.7 | 4.8 | 6.6 | 41.6 |
| German males | 3.8 | 9.9 | 6.6 | 7.9* | 6.7 | 4.3 | 4.1 | 43.3 |
| Italian females | 4.7 | 7.7 | 4.6 | 8.9 | 7.7* | 8.0 | 10.0** | 51.6 |
| Italian males | 4.8 | 9.7 | 4.2 | 8.9* | 6.5* | 6.9 | 6.4 | 47.6 |
| Moroccan general population | 4.4 | 7.3 | 2.4 | 6.1 | 9.8* | 3.7 | 10.0** | 43.6 |
| Polish general population | 6.9 | 8.9 | 2.6 | 9.8 | 7.8 | 4.6 | 5.2 | 45.8 |

*Scored by equation 2, because intake surpasses the recommendations. **Score of 10 was given for intakes of negative food groups that were lower than the upper levels. FVP, fruits, vegetables, and potatoes. Reprint with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

TABLE 8 Adherence scores to the PHD (APHD).

| Population | FVP | Cereals | Dairy | Protein equivalents | Fats | Sweets | Alcohol | Total score |
|---|---|---|---|---|---|---|---|---|
| Danish both genders | 8.7 | 9.4 | 7.2* | 8.6 | 7.9 | 8.4 | 6.1 | 56.3 |
| Danish females | 7.9 | 8.1 | 8.0* | 7.0 | 6.8 | 8.9 | 8.5 | 55.2 |
| Danish males | 9.5 | 9.3* | 6.5* | 9.6* | 9.1 | 8.2 | 5.2 | 57.4 |
| German females | 6.6 | 9.7* | 7.4 | 4.1 | 4.1 | 6.0 | 6.6 | 44.4 |
| German males | 6.3 | 7.8* | 8.2 | 6.8 | 5.8 | 5.3 | 4.1 | 44.4 |
| Italian females | 9.7 | 10.0* | 7.7 | 6.6 | 7.5 | 9.9 | 10.0** | 60.4 |
| Italian males | 8.9 | 7.8* | 7.0 | 8.3 | 8.9 | 8.5 | 6.4 | 55.9 |
| Moroccan general population | 9.5 | 4.6* | 6.5 | 8.1 | 8.4* | 4.6 | 10.0** | 51.7 |
| Polish general population | 6.9 | 7.7 | 6.2 | 7.6 | 6.7 | 5.7 | 5.2 | 46.0 |

*Scored by equation 2, because intake surpasses the recommendations. **Score of 10 was given for intakes of negative food groups that were lower than the upper levels. FVP, fruits, vegetables, and potatoes. Reprint with permission from Diet quality and sustainability in different countries and territories (Philippi Rosane, 2022).

## Sustainability of dietary patterns

The PHD is a global reference for a sustainable healthy diet that can be adapted to the local context and considers different populations, their food culture, nutritional status, and food availability (Willett et al., 2019). These characteristics made the PHD a reference diet for the five countries studied in the present study. Considering that the PHD is formulated to have lower environmental impacts, we can presume that populations with dietary patterns closer to the PHD, would have diets of a lower environmental burden than dietary patterns that differ considerably from the PHD. Therefore, we estimate that the populations with a higher APHD score would have a more sustainable diet (i.e., Italian females). Italians and Danes presented the highest scores for the APHD, mainly due to the highest scores for adequate intake of fruits, vegetables, and potatoes (FVP).

The "protein equivalents" group contains food groups with divergent environmental and health impacts. Red meats, especially beef, are the foods with the highest environmental burden in their production chain, due to land and water use, and greenhouse gasses emissions (Pradhan et al., 2013; Ritchie et al., 2018; Chai et al., 2019). Additionally, red meats and processed meats have been correlated to non-communicable diseases (NCDs) in different observational studies and reviews (Clark et al., 2019; Libera et al., 2021). Contrastingly, recent work with risk-benefit assessment on the substitution of red meats for pulses in Danish diets concluded that this substitution would improve public health due to a reduction of the burden of cardiovascular diseases and diabetes (Fabricius et al., 2021).

Ahead of the evaluation of adherence to FBDGs, sustainability aspects of the FBDGs were assessed for the countries studied in SysOrg; Denmark, Germany, Italy, Morocco, and Poland. In this previous study (Philippi Rosane, 2021), we focused on amounts of different food groups recommended as other pieces of advice related to sustainability, such as purchasing seasonal and local produce. The study concluded that the Moroccan Nutrition Guide (Ministère de la Santè, 2016) has the highest environmental impact among the five countries because it recommends the highest intake of meats. On the other hand, the Danish Dietary Guidelines (Danish Veterinarian and Food Administration, 2021) had the lowest environmental impact, with guidelines focusing on a plant-based diet with very low meat intake. In this context, Danish females presented the healthiest and more sustainable diets (Figure 1). Danes, however, eat more red meats and less legumes than recommended, while in Morocco they eat less meats than recommended and considerably more legumes than the European populations (Tables 5, 8; Supplementary material). These food groups differ significantly in their environmental impact.

Therefore, it is important to look not only at overall scores but at the intake and recommendations of the different food considering their impact on the environment and health. An assessment of the sustainability aspects of the FBDGs has also been seen as needed to complement the analysis of diet quality based on the FBDGs. Additionally, the use of APHD has been shown important as a complement to the FBDGs in the assessment of health and sustainability of dietary patterns.

## Strengths and limitations

Several strengths and limitations unfolded in the development of this dietary evaluation methodology. First, this methodology permits the application of tailored recommendations for the evaluation of each population, that is, their own FBDG. Second, this study also used a globally applicable reference diet (i.e., the PHD) to complement the cross-comparison. Third, another strength of the methods here proposed is the adaptability to different populations and recommendations. The comprehensive food groups as adherence scores to FBDGs can and should be adapted to the studied populations in future studies.

The following aspects were identified as limiting factors of the methodology. First, the merging of certain food groups, especially all meats and legumes. Second, the quality of foods within each group was not considered. Third, although most FBDGs recommend the intake of whole-grain cereals over refined grains (Philippi Rosane, 2021), this evaluation was not possible as their intake was not distinguished on any of the dietary intake surveys. Lastly, no statistical test to determine significant differences between adherence scores was applied, because each population was treated as one observation, and no variance in intake was collected.

The limitations caused by the use of comprehensive food groups can be minimized in the future if dietary intake and recommendations consider that certain foods cannot be grouped together, despite having somewhat similar roles in diets, as they differ in environmental and health impacts (e.g., meats and legumes, vegetables and potatoes). However, it should be noted that the comprehensive food groups method is flexible to assemble differences presented by the diet materials being analyzed. Therefore, in future studies, they should be adapted to the FBDGs and dietary intake under investigation.

Additionally, dietary intake data varied in methods used in the assessment of diets, as seen in Table 2. In Denmark, Germany, and Italy, intake was calculated based on individual assessment but using different tools (i.e., food frequency questionnaires (FFQs), interviews, 24 h recall, and food records). In contrast, in Morocco and Poland, consumption per capita was estimated based on household consumption. In Morocco, respondents to the national survey were asked to inform their total annual purchase of foods from the previous year (Haut Commissariat au Plan, 2016); in Poland, estimated consumption per household was calculated by monthly purchases and divided by the national average of residents per household (GUS, 2019). These differences are a limitation of our methodology since FFQ, 24 h food recall, and food records are more precise and trustworthy than household estimations and relaying on memory-based information (Henríquez-Sánchez et al., 2009; Archer et al., 2018).

## Conclusion

The present study proposes a diversified methodology to evaluate diet quality based on criteria of health and sustainability. To our knowledge, this study is a pioneer in evaluating dietary patterns according to FBDGs across different countries based on food groups. Our results show that using a combination of methods to compare dietary intake with national and global recommendations allows for cross-country comparisons. Due to the variability of data in the different countries, especially the use of different food groups, this study calls attention to the challenge of comparing different recommendations and dietary patterns. Therefore, the combination of the three methods developed can deliver an improved evaluation methodology of diets across different populations. Our cross-comparison methods for diet quality assessment (i.e., ADH2 and APHD) should not be used by themselves as the common food grouping (i.e., comprehensive food groups) impacted the adherence scores positively. This methodology can be useful for future evaluation of dietary patterns having health and environmental impact as quality aspects. Moreover, adherence to the FBDGs should be frequently assessed to guide nutrition campaigns and health policies.

Additionally, this study evidenced the need for assessing dietary intake aligned with the recommendations given in combination with separating foods according to their environmental and health impacts. To avoid wrong grouping and misleading scoring results, dietary intake surveys and recommendations should consider that certain foods cannot be grouped together. The reason is that food groups that currently are grouped together differ in environmental and health impacts (e.g., meats and legumes, vegetables and potatoes).

## 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

Conceptualization, methodology, and writing—original draft preparation: BP, LM, and SB. Data collection: BP, LM, RG-W, KK, DŚ-T, RK, LR, and YA. Data curation, formal analysis, and visualization: BP. Critical review and editing: LM, RG-W, KK, DŚ-T, RK, LR, YA, and SB. 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.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1147874/full#supplementary-material

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# Microencapsulated iron in food, techniques, coating material, efficiency, and sensory analysis: a review

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Iron deficiency in children and vulnerable people requires the intervention of effective emerging technologies to incorporate minerals into food, iron is an important micronutrient required by the human body to develop different functions. It's oxidation and susceptibility when added directly to food hinders its absorption, impairs sensory aspects, causing rejection by consumers. Currently, efficient, low cost, high productivity, better bioaccessibility and bioavailability, microencapsulation techniques have been developed. This review focuses on the study of the different methods and techniques of iron microencapsulation and its behavior in food fortification. The type of coating material, the efficiency, yield, bioaccessibility and bioavailability evaluated for each technique. It has been shown that the most used coating materials were maltodextrin, sodium alginate, gum arabic and whey protein; while the morphological characteristics and the release profile studied from 1995 to the present, on average were in the following order: Percentage of microencapsulation (85%), yield (76%), bioavailability (60%), and bioaccessibility (52%).; However, the sensory evaluations of some foods fortified with iron microcapsules denoted a metallic taste, color and smell were also detected, decreasing their level of acceptance. These findings suggest the need for further research to establish new protocols to fortify foods while maintaining their nutritional and sensory quality.

KEYWORDS

**functional foods, ferrous compounds, anemia, fortification, iron homeostasis**

## 1. Introduction

Iron (Fe) is a mineral and essential for human nutrition (Piskin et al., 2022). This item is present in the cells in the form of traces (Dasa and Abera, 2018), performing functions in the bone formation of the human body, production of energy Adenosine Triphosphate (ATP), synthesis of collagen and Red blood cells (Girelli et al., 2018); genetic differentiation and regulation synthesis of deoxyribonucleic acid (DNA); myelination of the mitochondrial and neuronal system (Gao et al., 2019). Approximately 66% of body iron is bound to hemoglobin, 8% to myoglobin, heme enzymes andiron-sulfur clusters. The rest (26%) is stored in the form of ferritin and hemosiderin in the liver (Figure 1; Ilich, 2020; Hall and Guyton, 2021; Salazar et al., 2021).

Food offers two sources of iron which are used by the human being; heme or heme iron, acquired by eating foods of animal origin with an absorption between 15 and 35% and non-heme iron, obtained from vegetables with an absorption of less than 10% (Skolmowska and Głąbska, 2019; Zhuo et al., 2019).

Iron deficiency in the body can contribute to the development of various diseases such as iron deficiency anemia (Cappellini et al., 2020), heart failure (Naito et al., 2021), kidney pathologies (Batchelor et al., 2020), intestinal inflammation (Nielsen et al., 2018), and development of neurobehavioral disorders (Pivina et al., 2019), 42% of children between 0 and 5 years of age, 33% of women of reproductive age and 40% of pregnant women suffer from iron deficiency anemia (El Bilbeisi et al., 2022), being Peru one of the countries whose population shows high rates of iron deficiency anemia (40% of children from 0 to 3 years of age) (Barrios Renteria et al., 2022), 28.3% of pregnant women between 15 and 18 years of age and late ages of 35 years and over, women who do not have health insurance (Espinola Sanchez et al., 2021).

The iron requirement in pregnant women is 27 mg/day (Cusick et al., 2018), while in children from 24 to 59 months it is 30 mg/day (Finkelstein et al., 2018). However, intestinal absorption of 1 to 2 mg/day is required for iron homeostasis to occur (Yiannikourides and Latunde-Dada, 2019). Worldwide there have been attempts to fortify commonly consumed foods with different concentrations of iron (Podder et al., 2018; Jahan et al., 2019; Pachón et al., 2021), nevertheless, the direct addition of the mineral in food negatively influences the organoleptic characteristics, mainly highlighting the metallic smell and taste, which causes limitations in its intake (Cheng et al., 2020). The use of microencapsulation to mask the sensory qualities of ferrous compounds has been extensively studied, generally using polymeric coating walls (Böger et al., 2021), which has allowed a correct bioavailability through the release and absorption in the gastrointestinal tract (Gaigher et al., 2022).

So far, several microencapsulation techniques, such as spray drying, spray cooling, ionic gelation, emulsification and freeze drying, have proven to be feasible for the microencapsulation of ferrous compounds (Bhosale et al., 2019). In the microencapsulation process, the encapsulation method, the wall material and the core constitute the factors that determine the product characteristics (particle size, shelf life, efficiency, performance, bioaccessibility, bioavailability and sensory aspects) (Gupta et al., 2014; Ballesteros et al., 2017; González Ortega et al., 2020). In this sense, this review aims to show the different methods and techniques of iron microencapsulation and its behavior in food fortification.

## 2. Microencapsulation

Microencapsulation is a coating method that confers the core (oxidizable substance, volatile additives, polyphenols, microorganisms), the necessary protection to prevent its loss or deterioration when exposed to various environmental factors (temperature, pH, oxygen), allowing it to keep the encapsulated material stable (Wang et al., 2017), reduces chemical interactions, prevents physicochemical changes and unpleasant sensory qualities (Pratap Singh et al., 2017); creating a barrier that allows controlled release and bioavailability of the active component at the time of ingestion, and facilitates transport and handling (Calderón Oliver and Ponce Alquicira, 2022; Figure 2) through a coating formed by proteins, carbohydrates and/or lipid compounds (Böger et al., 2021; Pratap-Singh and Leiva, 2021). Currently, there are different microencapsulation techniques that are low cost, easy to operate and highly efficient (Wardhani et al., 2021).

### 2.1. Spray drying

Among microencapsulation technologies, spray drying is considered one of the most important techniques to protect any excipient, including minerals, making use of a wide range of coating materials (Baldelli et al., 2023); the process is subjected to high temperatures (100–200°C) to shape a dry powder; the particle size ranging from 903.7 nm to 20 µm (Dueik and Diosady, 2017; Ligarda Samanez et al., 2022). Several studies have used spray drying to microencapsulate vitamins (Singh et al., 2018), probiotic microorganisms (Pratap Singh et al., 2017), animal blood cells (Ligarda Samanez et al., 2022), minerals (Cian et al., 2021), and other bioactive compounds (Ravichai and Muangrat, 2019). The advantages

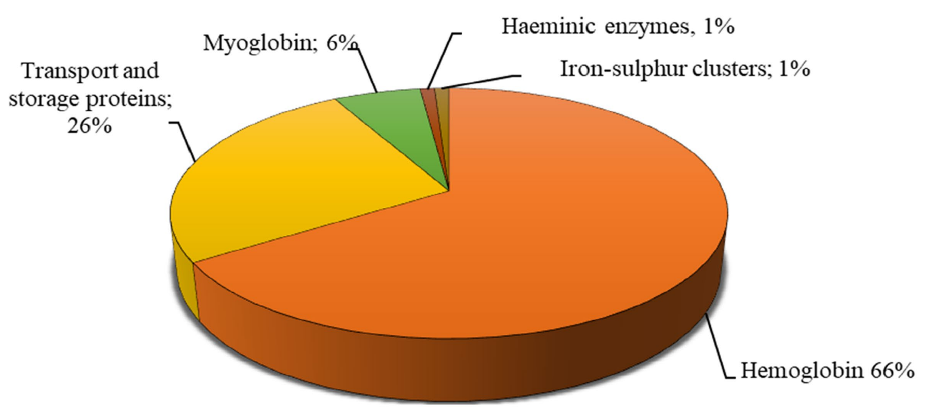


FIGURE 1
Iron proportions located in the human body. Adapted from Hall and Guyton (2021).

of this technique include ease of operation, low water activity of the final product, increased protection of some micronutrients such as iron against oxidation (Wardhani et al., 2021), particle distribution and loading capacity (Polekkad et al., 2021) and also improves iron release and absorption rate (Filiponi et al., 2019; Cian et al., 2021).

## 2.2. Ionic gelation

Ionic gelation is a favorable alternative to microencapsulate unstable compounds forming protein gel beads on their entire surface (Valenzuela et al., 2014). The composition of these pearls is due to the presence of sodium alginate and calcium chloride, which form a solid and stable structure with high encapsulation efficiency and soluble properties under neutral and alkaline pH conditions. These characteristics can be beneficial for the iron absorption because the duodenum has a similar pH (Yao et al., 2020). Perez-Moral et al. (2013) and Cengiz et al. (2019) performed simulations under gastrointestinal conditions under progressive release; in gastric conditions 45% was absorbed, a retention in the gastrointestinal tract of 43% and in duodenal conditions 12%; however, gels limit fortification in some food matrices (flours and powders) (Onsekizoglu Bagci and Gunasekaran, 2016) making it difficult to control the particle size, which usually varies between 3 and 5 nm (Katuwavila et al., 2016).

## 2.3. Single and double emulsion

Emulsion is a technique that does not require the intervention of high temperatures, has a controlled release and chemically stabilizes the encapsulation of nutrients and bioactive compounds (Choi et al., 2009; Ilyasoglu Buyukkestelli and El, 2019). There are two types of food emulsions, single emulsions with two delivery systems: oil-in-water (O/W) and water-in-oil (W/O) and double or hydrophilic emulsions composed of two inner (W1), outer (W2), and one oily (W1/O/W2) aqueous phases (Gupta et al., 2015). Single emulsion systems containing a core have a different sensory perception; one of the advantages of the simple O/W emulsion system is the core that is in direct contact with the palate, the oily phase acting as a filler causes the increase in taste perception by increasing the concentration of the core in the external aqueous phase, however, the same does not occur when working with the W/O system (Ilyasoglu Buyukkestelli and El, 2019) however, the most relevant drawback in this type of emulsions is the difficulty to turn into powder, but they do prove to be more stable than double emulsions during storage (Chang et al., 2016).

In double emulsions, the inner phase (W1) includes different solutes from the outer phase (W2), as well as the oily phase (O), which is composed of lipophilic substances, double emulsions have many applications in the food industry due to the multicompartmental system achieved by the single emulsion with suitable surfactants; some of their advantages are high efficiency during iron encapsulation, improvement of fatty acid profile and reduction of sugar/salt content in food (Ilyasoglu Buyukkestelli and Nehir El, 2019). Naktinienė et al. (2021) obtained an iron microencapsulation efficiency of 95% using polyglycerol polyricinoleate and whey protein dispersions; Ilyasoglu Buyukkestelli and Nehir El (2019) determined that the percentage bioaccessibility of ferric chloride in *in vitro* tests was 52.97% dispersing polyglycerol polyricinoleate in olive oil as the oily phase and whey protein isolate as the internal aqueous phase (W1) while sodium caseinate was used for the external aqueous phase (W2); this value is not constant as it increases while the dispersed phase decreases, causing the hydrolysis of sodium caseinate to act as an iron absorption enhancer; finally Katuwavila et al. (2016) found that the *in vitro* release rate of iron in alginate particles was 65% at 96 h under pH 7.4 compared to Churio and Valenzuela (2018) who observed the release of 31.8–37.4% under gastric conditions and around 100% of ferrous sulfate under intestinal conditions. Therefore, the double emulsion demonstrates better performance and can even microencapsulate chiral compounds.

## 2.4. Spray cooling

It is a technique characterized by its low cost and efficiency in the production of microparticles loaded with bioactive compounds, aromas and pigments (Figueiredo et al., 2022); its functions are based on the stabilization of ascorbic acid, vitamin D3, proanthoccocin and proanthocyanidins during storage (Carvalho et al., 2021). The technique consists of forming solid lipidic microcapsules in powder form of active sources of hydrophilic or hydrophobic origin, dispersed in dissolved lipid by spraying (Boesso et al., 2016; Sorita et al., 2021).

In one study, spray cooling has been used for the immobilization of ferrous sulfate heptahydrate using polyglycerol monostearate with a particle size of 2–5 μm, achieving an efficiency of 74% and a yield of 75% (Lee et al., 2003). However, to date there is limited research on the use of spray cooling to microencapsulate ferrous compounds, which may be related to the difficulty in finding lipid-based wall materials that meet the desired characteristics in terms of oxidative stability, food grade, melting point and storage stability (Figueiredo et al., 2022). For a better use of this technique, some additional operations could be considered and include the use of more sophisticated equipment to improve performance (Consoli et al., 2016).

## 2.5. Lyophilization

Freeze-drying is a technique that is performed at low temperatures, it manages to microencapsulate cells protecting them from bacteriophages and different factors during freezing and storage (Ribeiro Dias et al., 2017). It is a suitable technique for dehydration of heat-sensitive foods and microencapsulation (Desai and Park, 2005), its operations stabilize the material through four stages, firstly freezing of the core and wall material, sublimation of the lodged water avoiding friction with oxygen, desorption and finally storage (Ezhilarasi et al., 2013). Some of its advantages are related to low water activity, inhibiting the proliferation of microorganisms and improving the firmness of the microcapsules (Figueiredo et al., 2022); the technique is feasible to microencapsulate compounds intolerant to high temperatures, in addition to having a low oxidation rate (Kour et al., 2023); however, its disadvantages include the operation time (up to 20 h) and the production of porous spherical structures that cause rehydration of the microcapsules and, consequently, increased moisture (Karthik and Anandharamakrishnan, 2012). Wang et al. (2017) microencapsulated heme iron using maltodextrin, carboxymethyl cellulose and sodium caseinate achieving an efficiency of 98.64%.

Although all five techniques indicate different advantages over microcapsule formation, spray drying is considered the leading technique for protecting any excipient, including minerals, with its main advantage being the formation of microcapsules that meet physical properties (efficiency, moisture, bioavailability, and performance) that are stable in processing, packaging, distribution and storage; this is achieved by properly optimizing dry powders in formulations prior to producing microcapsules.

## 3. Coating or wall material

The wall material is one of the three essential factors involved in the microencapsulation process by acting as a protective agent of the core that covers the entire particle, which allows it to avoid reactions in food matrices (López Cruz et al., 2020), to form solutions with low viscosity at high concentrations, stable emulsions to form solid particles and with thermal stability (Đorđević et al., 2014; Mansour et al., 2020; Swami et al., 2021) withstand temperatures from 15 to 200°C to completely homogenize the samples (Jayalalitha et al., 2022), encapsulation is carried out continuously from a film on the particle (Karaaslan et al., 2021). Not all materials meet all the characteristics for encapsulation, for this reason, Gupta et al. (2015) and Polekkad et al. (2021) combined wall materials with similar origins and different properties to improve the efficiency and yield of microencapsulation.

The types of wall material used in microencapsulation are carbohydrates (Ribeiro Dias et al., 2017), proteins (Ying et al., 2016), and lipids (Zhu, 2017); they are characterized by being resistant to changes in temperature, oxygen and humidity, non-toxic, dissolve in acidic pH and are degraded by the endogenous microbiota of the small intestine (Pérez-Leonard et al., 2013), their stabilization depends on the time, speed of homogenization of the solutions and the ratio: core/wall material, if the amount of wall material is greater than that of the core, the microcapsules will be larger, spherical and with defined walls (Rocha-Selmi et al., 2013; Đorđević et al., 2014), the increase of solids in the formulation produces larger microparticles and lower bioavailability (Baldelli et al., 2023; Figure 2).

The most commonly used wall materials in the microencapsulation process are: maltodextrin, gum arabic, sodium alginate, whey protein and modified starch (Krokida, 2017); maltodextrin is a white hydrolyzed starch that is prepared by heating it until soluble, it is suitable due to its low viscosity at high concentrations, good protection against oxidation and good solubility (Premi and Sharma, 2017). However, its emulsification capacity is relatively low, which implies the need to combine it with other components to improve its yield and prolong its shelf life (Kanwal et al., 2022). Some edible gums (arabic, guar, gellan, tara, and xanthan) are composed of polysaccharides and are considered a suitable material for microencapsulation due to their low viscosity at high solid concentrations (Kang et al., 2019; Karrar et al., 2021), neutral taste (Chew et al., 2018), and emulsifying properties (Karaaslan et al., 2021), gum arabic is one of the most widely used carbohydrates in microencapsulation by retaining volatile compounds during the drying process (Rojas, 2019); being suitable for the formation of films and capsules of different active compounds such as minerals and anthocyanins (Pieczykolan and Kurek, 2019).

Sodium alginate is a biopolymer extracted from brown algae (Heckert Bastos et al., 2020), thermally stable (Singh et al., 2018), has the ability to form hydrogels and degrade at acidic pH (McKinney et al., 2022), presents thickening, stabilizing and film-forming properties (López Cruz et al., 2020). These characteristics allow it to be an important material in the microencapsulation of probiotics and minerals that allows its release in the digestive environment (Motalebi Moghanjougi et al., 2021). Whey proteins (β-lactoglobulin, α-lactalbumin) have the ability to interact and form aggregates, which determines their functional and technological properties; Can be used as a protective gel and form encapsulation coatings for bioactive compounds (Onsekizoglu Bagci and Gunasekaran, 2016; Pereira et al., 2017). Microencapsulation of iron in whey protein has shown high efficiency compared to other elements such as Zinc, due to the presence of a smaller atomic radius and the electronegativity of iron, which facilitates its binding with the carboxylic functional groups of the amino acids in the protein (Pratap-Singh and Leiva, 2021).

Modified starch can be obtained naturally or synthesized by chemical, physical or enzymatic processes, has adequate bioavailability, low viscosity, high solubility and low cost (Balakrishnan et al., 2021), and can be used in spray drying processes (Tatar Turan and Kahyaoglu, 2021). The use of modified starch in combination with maltodextrin and gum arabic was studied by Gupta et al. (2014) obtaining ferrous sulfate microcapsules with good stability and microencapsulation efficiency above 90%; however, for Ribeiro et al. (2020) the limitations of modified starch are related to its undesirable taste and poor aroma protection.

## 4. Iron microencapsulation efficiency and yield

The yield of the microencapsulation is determined by the proportion of the compound that makes up the microencapsulated nucleus in relation to the initial dispersion (Çam et al., 2014; Pratap Singh et al., 2017; Churio and Valenzuela, 2018; González Ortega et al., 2020). Yield is calculated using the following equation:

$$Y(\%) = \left( \frac{Final\ Microencapsulated\ on\ dry\ base\,(gr)}{wall\ material\,(gr) + encapsulated\ material\,(gr)} x100 \right)$$

Efficiency is the difference in the percentage of microencapsulated sample compared to the initial sample (Gupta et al., 2014; Shahidi Noghabi and Molaveisi, 2020; Castejón et al., 2021), the expression is shown below:

$$EE(\%) = \left( \frac{Initial\ material\,(gr) - non\ microencapsulated\ material\,(gr)}{Initial\ material\,(gr)} x100 \right)$$

Other authors use the division of the non-microencapsulated sample over the initial sample minus the unit (Zhang et al., 2007).

Microencapsulation percentages can vary due to three significant factors, the form of iron, the core/material ratio, and the technique used (Asghari Varzaneh et al., 2017; Calderón Oliver and Ponce Alquicira, 2022); it has been shown that the highest percentages of efficiency (>90%) were obtained using the methods

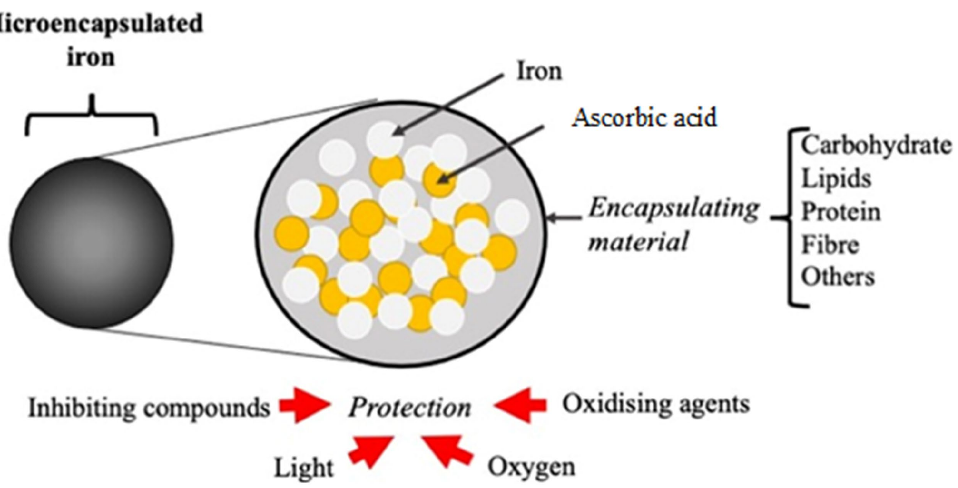


FIGURE 2
Model of a microcapsule of iron and ascorbic acid. Adapted from Calderón Oliver and Ponce Alquicira (2022).

of spray drying, double emulsion, ionic gelation and freeze-drying (Table 1).

Pratap Singh et al. (2017) observed that chitosan and Eudragit used as wall material in terms of efficiency (87–98%) and yield (68–75%), showed similar values; however, Eudragit demonstrated a yield (g microcapsules/L spray solution) 10 times higher than chitosan. Since the high viscosity of chitosan made it difficult to incorporate solids (1–2%) into the spray solution, unlike Eudragit (15–20% solids). Whey protein combined with Eudraguard has also been shown to be suitable for iron microencapsulation. The interaction of iron with the carboxylic groups of the amino acids of the whey proteins improves the protein-mineral affinity, this allows reaching efficiencies above 97% (Pratap-Singh and Leiva, 2021). Ligarda Samanez et al. (2022) used a wall material composed of potato starch and tara gum, allowing 94% efficiency in the microencapsulation of *Cavia Porcellus* erythrocytes.

The form of iron used in the microencapsulation process has implications for efficiency and yield. A study showed that when using iron gluconate and sulfate, 87 and 75% efficiency is obtained; however, when using iron citrate and chloride the efficiency is very low due to the formation of different particles and low solubility (Baldelli et al., 2023), it should be noted that ferrous sulfate is commonly used for food supplementation with iron, which generally leads to problems related to it is high reactivity, such as damage to the gastrointestinal mucosa due to free iron ions (Slivka et al., 1985) and also the development of undesirable color and flavor in the products in which it is applied. On the other hand, the operating conditions have a direct impact on the performance of microencapsulation. Increasing the inlet temperature in spray drying from 120 to 130°C improves the yield from 45 to 72% in iron microencapsulation using chitosan as the wall material, the iron loading density in the microcapsules was 25% for those conditions; however, increasing by 20°C would result in unacceptable iron oxidation (Dueik and Diosady, 2017). Furthermore, variations in spray drying performance may be subject to iron capsules sticking to the wall of the equipment or not passing through the filter, as well as very small particles being lost to the suction created by the vacuum pump (Dueik and Diosady, 2017; Pratap Singh et al., 2017).

Katuwavila et al. (2016) used the ionic gelation method to form nanoparticles with ferrous sulfate, ascorbic acid, calcium chloride, and sodium alginate; this system can function as a source of oral supply for the treatment of anemia, since it obtained 95% efficiency and 70% release at pH 6 (gastric conditions), the negative charge of sodium alginate is the driving force for the complexation of iron with the polymer, where chelation occurs between Fe2+ and carboxylate and hydroxyl molecules. Alginate beads loaded with ferrous sulfate of 0.76 mm in diameter were produced with 81% efficiency. It should be noted that the formation of beads can be stored for 45 days without altering the encapsulation efficiency (Cengiz et al., 2019). Wang et al. (2017) guaranteed 98.64% efficiency in the microencapsulation of blood with maltodextrin, sodium caseinate and carboxymethylcellulose (CMC) as emulsifier, because it preserves red blood cell proportions and guarantees the microbial quality of the blood. On the other hand, Mukhija et al. (2016) developed a microencapsulation by emulsions because it has a higher bioavailability in the release of iron, used sodium alginate as wall material and obtained 94% efficiency.

## 5. Bioaccessibility and bioavailability of iron microcapsules

The *in vitro* bioavailability and bioaccessibility as says of the microcapsules are models that simulate gastrointestinal conditions, which make it possible to determine if the microencapsulated iron is viable for the human body, given that the microencapsulated ferrous compounds must be adequately absorbed at the gastrointestinal level (Gaigher et al., 2022). Bioaccessibility represents the percentage of mineral that is soluble and ready to be absorbed in the digestive process. The main factors influencing this solubility are the chemical form of iron and the presence of inhibitors (Toledo Barbosa and Garcia Rojas, 2022). The *in vitro* bioaccessibility protocol is carried out by preparing salivary fluid, gastric and intestinal fluid and verifying enzymatic

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TABLE 1 Iron microencapsulation methods and their application in food.

| Microencapsulation method | | | | | Efficiency (%) /Yield (%) | Bioavailability (%)/ Bioaccessibility (%) | Royal iron end | Application matrix | Perception threshold | Considerations | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Technique | Coating materials | Core | Formulation: coating material/core | Particle size | | | | | | | |
| Spray drying | Chitosan | Ferrous sulfate heptahydrate | 1.5%: 1%: 20% CH:AC:FS | 10 µm | NR/72 | NR/NR | NR | Coarse salt | NR | >25% iron inhibits chitosan release | Dueik and Diosady (2017) |
| Spray drying | Chitosan | Ferrous sulfate heptahydrate | 1%:1%:15% (w/w) CH/AC: FS | 3 and 5 µm | 98/72 | 89/NR | 2.8–5.3% (w/w) | NR | NR | NR | Pratap Singh et al. (2017) |
| Spray drying | Eudagrit | Ferrous sulfate heptahydrate | 57.1:5.7:8.6:28.5 EPO:AT:AE:T (15% TSS in water)40% FS | 3 and 5 µm | 94/74 | 99/NR | 9.6% (w/w) | NR | NR | > Eudragit, improves iron payload. | Pratap Singh et al. (2017) |
| Spray drying | Maltodextrin and polydextrose | Ferrous sulfate heptahydrate and ascorbic acid | 80:20(p/p) MD/PD 1:1.6 (w/w) FS/AA 77/23 (p/p) wall and core | 6.85 µm | 83.9/92.5 | 34.85/NR | 13.76 mg of iron/g | NR | NR | 25% more bioavailability | Filiponi et al. (2019) |
| Spray drying | Whey protein and Eudraguard | Ferrous sulfate heptahydrate | 12:4:0.6 gr/gr/gr WPI/EPO/FS | 126 p.m. | 97.3/74 | NR/NR | 0.6 mg/L | NR | NR | Iron microcapsules are resistant to intestinal conditions, 100% iron was released in 45 min. | Pratap-Singh and Leiva (2021) |
| Spray drying | Sodium alginate degraded by cellulase | Ferrous sulfate heptahydrate | 3%:350 ppm:10mgSA/C/FS | 6.5 µm | 87.2/NR | NR/NR | 10 ppm | NR | NR | Alginate protects iron from oxidation up to 98.3% after 120 days. | Wardhani et al. (2021) |
| Spray drying | Locust bean gum, maltodextrin and beer protein concentrate | Ferrous sulfate heptahydrate and ascorbic acid | 17.2/11/13/1.7 gr/gr/gr/gr BSG/LBG/MD 0.9:1 AA/FS | 12.9 µm | 94.5/NR | NR/50.8 | 0.45 g of iron in 100gr | NR | NR | BSG proteins maintain soluble iron at the gastrointestinal level | Cian et al. (2021) |
| Spray drying | Tara gum potato starch | *Cavia porcellus* erythrocytes | 4%/1%/20% PF/GT/EC | 900.2 nm | 58.73/NR | 94.71/NR | 2.05 mg of iron/g | NR | NR | The nanometer size can precipitate in colloidal solutions. | Ligarda Samanez et al. (2022) |

*(Continued)*

TABLE 1 (Continued)

| Microencapsulation method | | | | | Efficiency (%) /Yield (%) | Bioavailability (%)/ Bioaccessibility (%) | Royal iron end | Application matrix | Perception threshold | Considerations | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Technique | Coating materials | Core | Formulation: coating material/core | Particle size | | | | | | | |
| Spray drying | Hydroxypropyl-methylcellulose and maltodextrin | Ferrous sulfate heptahydrate | 0.9/0.9/0.9 HP/MD/FS | 5.5 μm | 85/NR | 80/NR | NR | NR | NR | The low solubility of iron citrate and chloride causes low efficiency. | Baldelli et al. (2023) |
| Spray cooling | Polyglycerol monostearate | Ferrous sulfate heptahydrate | 5:50:1 (g/g/ml) PGMS/H2O/FS | 2–5 μm | 74/75 | NR/NR | NR | Pasteurized milk | Flavor and color is maintained for 24 h. On day 5 the metallic taste is intense. | Its intake could cause poisoning. | Kim et al. (2003) |
| Emulsion | Sodium alginate and modified starch | Ferrous sulfate heptahydrate and ascorbic acid | 1. Emulsion 1 (W1) 1.5:100:0.798 SA/H2O/FS Emulsion O 1:5 W1/SO | NR | 74.85/– | NR | 10 mg/L of milk | Strawberry cow and buffalo milk (1:1) (3% fat and 8% non-fat solids) | There are no differences with respect to unfortified milk. | The higher concentration of CaCl2 decreases the efficiency of the microcapsules. | Gupta et al. (2015) |
| Emulsion | Whey protein, sunflower oil and sodium alginate | Ferrous sulfate heptahydrate | Emulsion 1 (W1) 20gr:8gr WPI/FS Emulsion O 1:4:5 W1/AS/SO | NR | NR/NR | NR/NR | 80 mg of iron encapsulated in yogurt | Yogurt | <80 mg/L does not affect general preference, appearance, or texture. | Iron microcapsules do not affect the viability of probiotic bacteria. | Subash et al. (2015) |
| Double emulsion and spray drying | Palm oil and whey protein | Ferrous sulfate heptahydrate | Emulsion 1 (W1) 6:4 PO/FS Emulsion 2 7.5:2.5 WPI/W1 | 6 μm | 93.63/NR | NR/NR | 0.63 mg/200 mL of milk | Pasteurized milk | At concentrations of 0.3–0.7% (p/v) changes in color and flavor are perceived. | The concentration of 01–2% did not modify the values of thiobarbituric acid (in charge of fat oxidation). | Chang et al. (2016) |

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TABLE 1 (Continued)

| Microencapsulation method | | | | | Efficiency (%) /Yield (%) | Bioavailability (%)/ Bioaccessibility (%) | Royal iron end | Application matrix | Perception threshold | Considerations | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Technique | Coating materials | Core | Formulation: coating material/core | Particle size | | | | | | | |
| Double emulsion | Polyglycerol sodium chloride polyricinoleate (NaCl) | Ferrous sulfate heptahydrate | Emulsion 1 (W1) 5.84 mg/L:1150 mg/L NaCl/FS Emulsion O 6% (w/w) Emulsion 2 (W2) 4%:0.02% (w/v) Tween80/ sodium azide Double emulsion 20 g:80 g W1:O 40 g:60 g W1/O:W2 | 19.01 μm | NR/NR | 37.28 to 42.94/NR | 1,150 mg per liter of yogurt | Yogurt | NR | The release rate of iron in the yogurt increased after 7 days. | Hashem Hosseini et al. (2019) |
| Double emulsion | Sodium chloride, polyglycerol polyricinoleate, and whey protein | Ferrous sulfate heptahydrate | Emulsion 1 (W1) 5 g/kg:6 g/Kg NaCl/FS Emulsion O 40 g/Kg PGPR Emulsion 2 (W2) 5 g/Kg WPI Double emulsion 20:80 W1:O 40 g:60gW1/O:W2 | 74 μm | 52/NR | NR/NR | 14 mg/100 g | NR | NR | it had great physical and oxidative stability and iron entrapment efficiency. | Naktinienė et al. (2021) |
| Double emulsion | Whey protein, polyglycerol polyricinoleate, tara gum, and sucrose | Ferrous sulfate heptahydrate | Emulsion 1 (W1/O) 5%p/p:0.8%p/p PGPR:FS Emulsion 2 (W2) 12:0.8:2%p/p:%p/p:%p/p WPI:TG:S double emulsion 1:4 W1/O:W2 | 757.1 nm | 96.95/NR | Adults NR/49.54 ChildrenN.R/39.71 | NR | NR | NR | Stable microcapsules with high bioavailability were created, it is a potential alternative to use in liquid foods. | Toledo Barbosa and Garcia Rojas (2022) |
| Ionic gelation | Sodium alginate | Dried blood cells | 1:5 p/p SA/DBC | 1.13 nm | 75.7/NR | NR/NR | 367.9 μg/g | NR | NR | NR | Valenzuela et al. (2014) |

*(Continued)*

TABLE 1 (Continued)

| Microencapsulation method | | | | | Efficiency (%) /Yield (%) | Bioavailability (%)/ Bioaccessibility (%) | Royal iron end | Application matrix | Perception threshold | Considerations | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Technique | Coating materials | Core | Formulation: coating material/core | Particle size | | | | | | | |
| Cold gelation | whey milk protein | Ferrous sulfate heptahydrate | 6.8% (w/w): 60 mg WPI: FS | 1 mm | NR/NR | NR/NR | 60 mg in a liter of yogurt | Yogurt | They retain their sensory quality for 14 days | On day 15, it showed an uncharacteristic yellow color, progressively each sensory quality was deteriorating. | Onsekizoglu Bagci and Gunasekaran (2016) |
| Ionic gelation | Sodium alginate and calcium chloride | Ferrous sulfate heptahydrate and ascorbic acid | SF:AA = CORE 15/1 0.3p/v:40:50 SA: CaCl2:CORE | 20 ± 6 nm | 95/– | NR/NR | 1% w/w by weight of alginate | NR | NR | 70% release was at pH 6. | Katuwavila et al. (2016) |
| Ionic gelation | Sodium alginate | Ferrous sulfate heptahydrate and L-ascorbic acid, *L. acidophilus* | 3/4/15/80%:%:mg:mg LA/SA/FS/AA | NR | NR/71–92 | NR/NR | NR | NR | It does not show a significant effect. | The lower the viscosity of the sodium alginate, the higher the efficiency of the bacteria. | Kumar et al. (2017) |
| Ionic gelation | Maltodextrin | Ferrous sulfate | 40%(p/v):30%(p/v) MD: FS | 959 µm | NR/57 | NR/NR | 78.4 mg/g | NR | NR | Maltodextrin is easily dispersed and dissolved in water, high release of iron in gastric media. | Churio et al. (2018) |
| Ionic gelation | Sodium alginate | Ferrous sulfate | 30gr:10grSA/FS | 820 µm | 81/NR | NR/NR | 2.98% by weight | NR | NR | Effective inhibition of iron pro-oxidant activity. | Cengiz et al. (2019) |
| Internal ionic gelation | Sodium alginate and caseinate, whey protein | Ferrous fumarate | 15/52.5/30/2.5 gr/gr/gr/gr SA/WPI/SC/FF | 51.2 µm | 97.2/67 | NR/NR | 28.6 mg iron/100 g | NR | NR | The gel beads prevent the breakdown of hydroperoxides and the development of peroxide lipid radicals | Yao et al. (2020) |

*(Continued)*

TABLE 1 (Continued)

| Microencapsulation method | | | | | Efficiency (%) /Yield (%) | Bioavailability (%)/ Bioaccessibility (%) | Royal iron end | Application matrix | Perception threshold | Considerations | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Technique | Coating materials | Core | Formulation: coating material/core | Particle size | | | | | | | |
| Solvent evaporation | Arabic gum, maltodextrin, and modified starch | Ferrous sulfate and ascorbic acid | 4:1:1:10 GA/MD/MS/ H2O Lamount of solids (6 gr) 15:1FS/AA | 15.5 µm | 91.58/NR | NR/63.7 | 25 ppm iron | Strawberry cow and buffalo milk (1:1) | Mouthfeel showed changes on day 3. Color, flavor and appearance from day 5. | The low concentration of alcohol decreases the retention capacity, causes a slow release of water and longer dehydration time. | Gupta et al. (2014) |
| Solvent evaporation | Sodium alginate and calcium chloride | Ferrous sulfate heptahydrate | 2:2 (COAT)%w/v: %w/v SA:CaCl2 1:1 COAT: FS | 209 ± 2.5 µm | 67/NR | NR/NR | NR | NR | NR | The microcapsules were uniform, hard and stable for 30 days. | Al Gawhari (2016) |
| Lyophilization | Sodium caseinate, maltodextrin, and carboxymethylcellulose | Goose blood (Anser) | 3.76:1.82:1.86:40.8 SC:MD:CMC:H2O3:20GB:H2O | 50 µm | 98.64/NR | NR/NR | 0.90 mg/g | NR | NR | NR | Wang et al. (2017) |
| Does not specify the method | Vegetable fats | Ferrous sulfate heptahydrate and ascorbic acid | NR/NR | 0.8 µm | NR/NR | NR/NR | 68.7 mg iron/ 80 mg microcapsules | Feta Cheese | No off flavors detected | Thiobarbituric acid values were significantly lower compared to non-encapsulated iron. | Jalili (2016) |
| Does not specify the method | Hydrogenated palm oil | Ferrous sulfate heptahydrate | NR/NR | 700–1,000 µm | 91/– | NR/NR | 0.17 mg/g of cheese | Cow's Milk Cheddar Cheese | The taste, color and smell could not be completely masked. | The lipophilic coating material on the small particles interacts better with the fat in the cheese. | Arce and Ustunol (2018) |

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TABLE 1 (Continued)

| Microencapsulation method | | | | | Efficiency (%) /Yield (%) | Bioavailability (%)/ Bioaccessibility (%) | Royal iron end | Application matrix | Perception threshold | Considerations | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Technique | Coating materials | Core | Formulation: coating material/core | Particle size | | | | | | | |
| Direct addition | Non-hydrogenated vegetable fat | Ferrous sulfate heptahydrate | NR/NR | 700–800 μm | 73.5/– | NR/NR | Total iron in cheese 0.9482 per kg | Goat's Milk Cheddar Cheese | The sample presented the lowest values at the sensory level. | Fortified cheeses obtained greater hardness due to proteolysis. | Siddique and Park (2019) |
| Direct addition | NR | Ferrous sulfate heptahydrate | NR | NR | NR/NR | NR/NR | 2.5, 5 mg of iron in a liter | Yogurt | Yogurt sensory properties intact. | Iron has the ability to extend and form bridges with casein. | Jasim and Al-Saadi (2020) |
| Direct addition | NR | Powdered swine blood | NR | 30 μm | NR/NR | NR/NR | 4.53 mg in 100 g of Yogurt | Yogurt | Formulas with 10% pig blood meal were acceptable. | Lactic acid fermentation increases iron availability by absorbing between 20 and 30% heme iron. | Huaraca Aparco et al. (2021) |
| Direct addition | NR | AprosanTM (Powdered Porcine Blood) PPB | 2.0/6.76 g/mg Chocolate /PPB | NR | NR/NR | NR/NR | 21.82% (6.76 mg) of iron in a liter of milk | Chocolate milk | From 20% iron an unpleasant metallic taste is obtained | Microencapsulation prevents microbial proliferation | García et al. (2022) |

SF, ferrous sulfate; FF, ferrous fumarate; EC, *Cavia porcellus* erythrocytes; GB, Goose blood; DBC, dried blood cells; LA, L-acidophilus; AA, Ascorbic acid; SA, Sodium alginate; SC, Sodium caseinate; MD, Maltodextrin; GA, gum arabic; TG, tara gum; WPI, Whey protein; PGPR, Polyglycerol Polyricinoleate; PGMS, Polyglycerol Monostearate; HP, Hydroxypropyl-methylcellulose; CH, Chitosan; PS, Polydextrose; MS, modified starch; NaCl, Sodium Chloride; CaCl, Calcium Chloride; C, Cellulose; BSG, Beer Protein Concentrate; HS, Sucrose; PO, palm oil; SO, Sunflower oil; PF, potato starch; EPO, Eudragit; AC, Acetic acid; AT, Tartaric acid; EA, stearic acid; T, Talc; NR, Not reported. Emulsion O, Oily phase; Emulsion 1, Internal aqueous phase; Emulsion 2, External aqueous phase.

activity, this method is called "*In vitro* static simulation of gastrointestinal digestion of food" (INFOGEST 2.0) designed by Brodkorb et al. (2019). Ilyasoglu Buyukkestelli and Nehir El (2019) evaluated the bioaccessibility in the microencapsulation of iron in the technique (W/O:W) resulting in 52.97%; by increasing the dose of iron in the first phase and keeping the second phase constant, bioaccessibility can be improved. Cian et al. (2021) used brewery grain protein concentrate (BSG-PC), locust bean gum and maltodextrin to microencapsulate ferrous sulfate, determined the bioaccessibility percentage using the protocol of Miller et al. (1981) and Drago et al. (2005), preparing controlled pH media and adding the optimization process, obtaining as a result a high percentage of bioaccessibility. This result was favored by the presence of ascorbic acid (AA) in the microcapsules, by reducing the concentration of BSG-PC, the AA/Fe molar ratio increased, giving rise to the action as a chelating agent of AA on Fe. This effect, it was also provided by BSG-PC when its concentration increased. The reducing action of BSG-PC on Fe favored the increase in bioaccessibility values.

Toledo Barbosa and Garcia Rojas (2022) adapted the simulation of digestive intake of infants and adults, resulting in two acceptable bioaccessibility values, for each case they applied the INFOGEST method under biaccessibility conditions in adults and Ménard et al. (2018) in children, obtaining 49.54% while in adults it was 39.71%; the data obtained by the mentioned methods are replaced in the following formula to determine the percentage of bioaccessibility (BC).

$$BC(\%) = \frac{Iron\ content\ before\ digestion\ in\,vitro}{Iron\ content\ after\ digestion\ in\,vitro} x100$$

Differences between adults and children can be explained by pH values and enzyme activity that affected digestion (Toledo Barbosa and Garcia Rojas, 2022).

Bioavailability is determined as the percentage of ingested metal free for the metabolic process (Blanco-Rojo and Vaquero, 2019), the most widely used method is transport by CaCo-2 cells (human colon adenocarcinoma cells), used by Filiponi et al. (2019) in the microencapsulation of ferrous sulfate with maltodextrin and polydextrose, obtaining as a result a bioavailability of 56.21%; Christides et al. (2018) evaluated the availability of iron in infants supplemented with prebiotics by adding a heat treatment to the mentioned method, the formulation with the best bioavailability had a 4:1 ratio. Hemalatha et al. (2007) evaluated the bioavailability of zinc and iron in food cereals, the result with zinc was not as expected, being decreased by the heat treatments used, while with iron the results increased significantly. The direct incorporation of iron in dairy foods stimulates a reaction with milk proteins and fats, lower bioavailability and organoleptic problems (Naktinienė et al., 2021). Iron oxidation is prevented by microencapsulation by forming an impermeable membrane as a barrier to oxygen expansion, covering unacceptable tastes and odors of iron salts, increasing their bioavailability (Gupta et al., 2014). The percentage of bioavailability *in vitro* in the investigation of Hashem Hosseini et al. (2019) it is found from 37.28 to 42.94% by double emulsion; 63.78% ± 0.23 was the result of microencapsulated iron fortification by Gupta et al. (2014) and Baldelli et al. (2023) obtained 80% bioavailability in microparticles of ferrous sulfate with hydroxypropylmethylcellulose in the experiment of Mukhija et al. (2016) they obtained 90.4% in an iron/alginate/oil emulsion; a balance must be considered between the percentage of solids, an increase of 80% solids increases the particle size by 30% and improves its spherical shape, however, it decreases the bioavailability to 25% because the large microcapsules need more time to dissolve. It should be noted that iron microcapsules are less affected by the presence of inhibitors, so the bioavailability of iron is high (Gupta et al., 2014). Both the AA and the peptide-metal complexes in the microparticles induced a higher iron absorption and, therefore, could be a good alternative to obtain more bioavailable iron (Filiponi et al., 2019). However, poor encapsulation has been correlated with a faster release rate and lower bioavailability. For example, in dairy products, free iron interacts with casein, leading to the formation of insoluble iron in the gastrointestinal tract, which reduces it's bioavailability (Subash et al., 2015). For this reason, it is important that the coating materials have a high molecular weight, this is not only essential for its influence on the efficiency of microencapsulation, but also for its implication on the release rate of encapsulated iron (Baldelli et al., 2023).

$$BD(\%) = \frac{CC.of\ \ iron\ worked\ in\ digestion - Iron\ content\ C.White}{Content\ of\ \ iron\ released\ in\ the\ digested\ emulsion} x100$$

CC = Quantity of Cells.
C. = Cells.

## 6. Success rate in iron microencapsulation

Iron as a mineral has an undesirable sensory perception for its intake, despite the fact that it is vital for human health. Investigations focused on the use of technologies to modify the sensory aspects of the different forms of iron without impairing their nutritional value have been registered. Iron microencapsulation is a technology that presents the opportunity to prevent iron deficiency, increase bioavailability, avoid the perception of metallic taste, and protect from chemical reactions that occur when in contact with food matrices. The statistical data registered by the National Library of Medicine (PubMed) indicate that the iron microencapsulation method began in 1971, but it was deepened with the study of Boccio et al. (1995), when studying a new procedure to enrich liquid milk and its derivatives with iron; using ferrous sulfate as the core and soybean lecithin as the wall material, pointed out that iron absorption is influenced by some food additives, such as the presence of 10% (w/v) of cocoa, which manages to increase iron absorption, while that tea and coffee decrease these values. From this, 113 publications have been registered until the year 2023, regarding the microencapsulation of iron.

Microcapsules have numerous parameters that are studied through morphological tests and release profiles, which determine their stability and safety (Zárate-Hernández et al., 2021). The present study considers that the percentage of success in iron microencapsulation is reflected in the average value of efficiency, yield, bioavailability and bioaccessibility, parameters listed in Table 2.

Studies carried out in the years 1995–2001 used the vacuum drying method to microencapsulate ferrous sulfate in soy lecithin; in order to fortify milk and evaluate its bioavailability through the prophylactic-preventive method (Lysionek et al., 2001); the results varied from 7 to 12%, this was due to the iron-casein interaction. This is a phosphoprotein that oxidizes Fe2+ to Fe3+, producing insoluble compounds in milk, which produce an inhibitory effect on iron absorption; mechanism reflected in a low bioavailability around 12% (Boccio et al., 1998). However, in recent years new research has emerged that has allowed the microencapsulation process to be improved, obtaining better efficiency indicators.

## 7. Iron absorpion in the human body

The iron present in the human body fulfills three main functions, it forms myoglobin, hemoglobin, enzymes and hormones; receives and stores serum ferritin and hemosiderin when their input is decreased and transports oxygen to the tissues of the human body (Shubham et al., 2020).

The metabolism and absorption of iron is represented in Figure 3. The iron acquired by food intake is absorbed by the duodenum and proximal jejunum, giving off 3 phases of absorption: luminal, mucosal, and body (Dasa and Abera, 2018). The first phase is responsible for processing food with gastric acid, causing the release of iron from the microcapsules in its bivalent or ferrous form. The pH of gastric acid converts ferric or trivalent iron (Fe3+) to ferrous iron (Fe2+) through duodenal cytochrome B enzyme in the brush border of enterocytes (Lo et al., 2022).

The second phase occurs in the intestinal mucosa located in the upper part of the yuoyene, iron enters through the apical membrane through the divalent metal transporter I (DMTI); with in enterocytes Fe2+ is stored as ferritin and transported from the basolateral membrane via the transmembrane protein ferroportin 1 (Dasa and Abera, 2018). The body phase includes the process of transport and storage of the mineral, for the transport of iron at the body level, Fe2+ is converted to Fe3+ by means of the hephaestin protein found in the ferroportin transmembrane; iron circulates through transferrin which occupies 20–40% of the saturation percentage of transferrin; finally transported by bone marrow and storage organs (liver) (Hall and Guyton, 2021).

TABLE 2 Success rate of published trials/experiments.

| Results | Number of investigations | Average % |
|---|---|---|
| Efficiency | 26 | 85 |
| Yield | 14 | 76.45 |
| Bioavailability | 22 | 59.77 |
| Bioaccessibility | 5 | 52 |

The number of investigations that have determined the four parameters and the average success they obtained when microencapsulating iron are described.

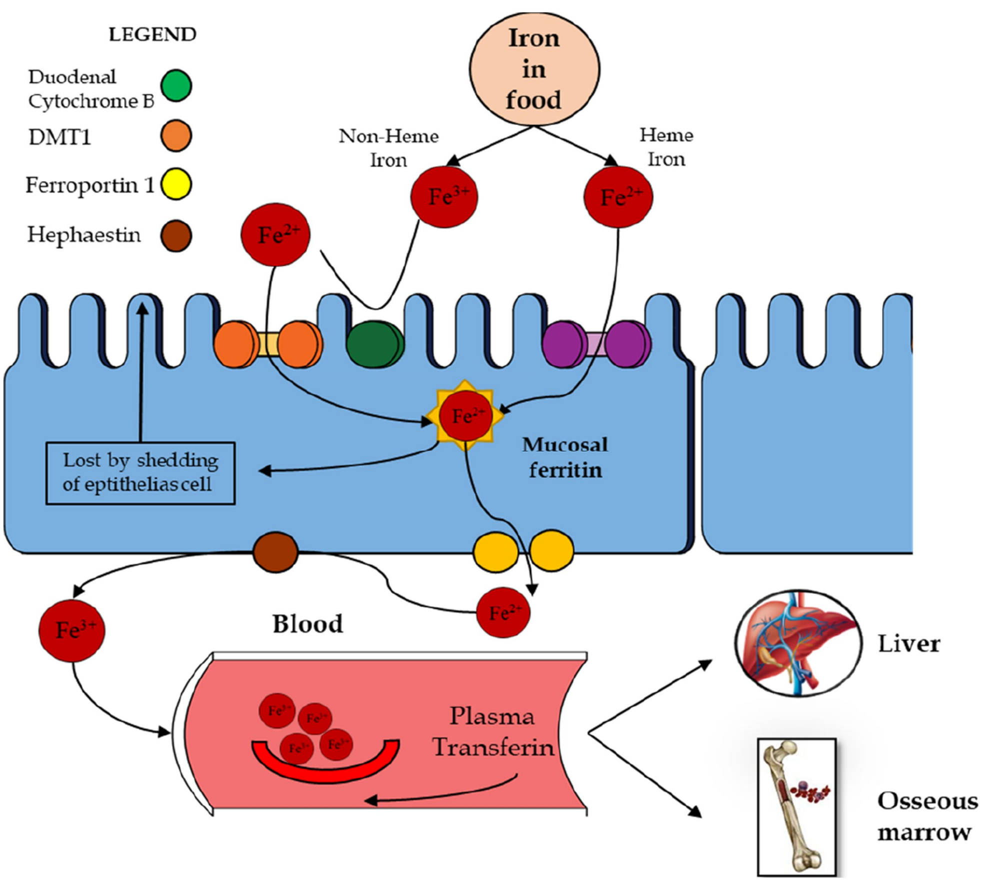


FIGURE 3
Iron absorption and metabolism. Adapted by Shubham et al. (2020).

## 8. Shelf life and viability of iron microcapsules

The useful life and/or stability of microcapsules is evaluated according to the technique and wall material of the microencapsulation. Hae Soo (2014) describes that temperature is one of the factors that intervenes in the oxidation of lipids, influencing the acceptance of the product, therefore the microparticles of oil and fat (simple emulsions) deteriorate, reducing their useful life, the nutritional value and causing substances toxic. Toledo Barbosa and Garcia Rojas (2022) used the double emulsion technique made with whey protein and polyglycerin, demonstrating that after 7 days of storage at 25°C there is a significant increase in particle size and apparent viscosity. Naktinienė et al. (2021) confirmed that the reaction described above could be due to the interaction of the hydroxyls of polysorbate molecules with iron in the external phase and the formation of networks, causing the increase in viscosity, in addition, the diffusion of water from the external to the internal phase causes growth. Osmotic causing the particle size. Lee et al. (2011) verified that microencapsulated iron salts with whey protein and oil in water are easily modified to become dry powder through the intervention of a vacuum evaporator, prolonging their useful life and effective iron enrichment in milk. Baldelli et al. (2023) analyzed the stability of iron microcapsules obtained by spray drying, storing 20 mg at 21°C and 35% humidity in the environment. The estimated percentage in dry powders is 15%; however, at 90 days the values exceeded the moisture index. García et al. (2022) fortified chocolate milk with heat-treated non-microencapsulated heme iron, with a concentration of 9.3 mg/kg$^{-1}$ of iron, was accepted by the Institute of Medicine to supplement iron levels in children 8–11 years of age (8 mg/day), the sensory level was acceptable but its useful life lasted only 5 days because the product exceeded the estimated percentage of aerobic mesophiles (50,000 CFU/mL$^{-1}$) and evidenced organoleptic problems; Huaraca Aparco et al. (2021) confirmed the sensory acceptance and the stability of the physicochemical parameters when adding heme iron to yogurt; In comparison with Subash et al. (2015) who demonstrated its viability in compound yogurt with probiotic bacteria by adding 80 mg of ferrous sulfate microcapsules during 20 days of storage at 4°C, likewise Figueiredo et al. (2022) mentioned that there is no alteration in the intestinal microflora when ingesting yogurt fortified with iron microcapsules. Leiva Arrieta (2020) demonstrated that the absorption of iron by Caco-2 cells presents a cell viability greater than 75% in exposure at the intestinal level. Finally, Sadiq and Doosh (2019) they used sodium alginate and ferrous sulfate subjected to spray drying, their evaluation was carried out in yogurt for 21 days at 5°C, the initial stability values were 87.02 and 86.83%; while at 21 days they decreased to 86.62 and 86.62% respectively, decreasing 0.20%, there was no significant difference between the physicochemical properties, but low values of the degree of acidity and peroxide index.

## 9. Sensory analysis

Sensory characteristics condition the acceptance or rejection of a food by consumers (Mihafu et al., 2020; Böger et al., 2021). When the food is exposed to factors such as the presence of oxygen, light and changes in pH, chemical reactions of degradation and oxidation are generated between the components of the food; that negatively affect the sensory qualities and absorption rate of nutrients, including minerals (Prichapan et al., 2018); which conditions the direct addition of compounds that improve the nutritional and bioactive properties of food (Halahlah et al., 2022). Mainly, foods enriched with microencapsulated compounds must undergo a sensory evaluation in order to mask foreign aromas and flavors that may be found in the product (Dias et al., 2015).

In the case of the iodized salt fortified with micronutrients such as iron, folic acid, vitamin B 12 and zinc was organoleptically acceptable in terms of appearance, aroma, taste, texture and aftertaste, except for color, where significant differences were found (Puri et al., 2022).

In contrast, the use of a high-quality coating for iron, which, together with iodine in the fortification of a salt, can constitute a physical barrier between the two and could significantly improve their efficacy (retention level and sensory attributes) (Vatandoust et al., 2021).

Kim et al. (2003) they fortified pasteurized milk with microencapsulated ferrous sulfate, after 24 h of storage, the color and flavor presented a slight change, while on the fifth day the perception of the metallic flavor was intense; Gupta et al. (2014) found changes in color, taste, smell and appearance in general on the fifth day, while the sensation in the mouth was perceived on the third day. On the other hand, Gupta et al. (2015) fortified milk by adding microencapsulated ferrous sulfate in a concentration of 10 mg/L, the result was feasible as no significant differences were found between control milk (without fortification) and fortified milk. In the same line, Chang et al. (2016) fortified milk with iron adding 0.1–0.2% demonstrating that under these conditions no reactions of thiobarbituric acid (responsible for fat oxidation) were observed. Besides, García et al. (2022) incorporated 6.76 mg of powdered porcine blood into 1 L of chocolate milk, which until the fourth day of storage, the sensory characteristics remained acceptable; however, when this concentration was exceeded, the unpleasant metallic taste in the product was predominant. In the fortification of Cheddar cheese with microencapsulated ferrous sulfate, Arce and Ustunol (2018) and Siddique and Park (2019) they failed to mask the taste, color and smell of iron; however, in the study of Jalili (2016), the feta cheese did not present unpleasant metallic flavors.

For Onsekizoglu Bagci and Gunasekaran (2016), adding concentrations <60 mg of microencapsulated iron for each liter of yogurt, do not affect the sensory qualities of the product for 14 days; in the same way Jasim and Al-Saadi (2020) evaluated that there are no alterations when adding 2.5 and 5 mg of ferrous sulfate directly. Huaraca Aparco et al. (2021) They found that the addition of 10% pig blood meal in yogurt favors acceptance at the sensory level; It should be noted that the fermentation produced by lactic bacteria increases the levels of iron bioavailability by 20 to 30%, which makes yogurt a favorable vehicle for fortification with iron microcapsules.

## 10. Limitations found in the reviewed studies

The main limitations found in the different studies was the need to take measurements in the iron suspension formulation, data necessary to identify the estimated values in the particle formation process (Baldelli et al., 2023). Some coating materials used have limited solubility in hot water, when it manages to disperse it swells and forms a highly viscous solution, unpleasant odor, non-Newtonian behavior and rapid biodegradability, such is the case of tamarind gum

(Halahlah et al., 2022). Some methods to determine bioavailability have not yet been fully studied, such as the Caco-2 cell model, since other organs (liver) are involved in its regulation in its absorption process and are not considered in the studies (Gaigher et al., 2022). So far, the perception of sensory attributes in foods fortified with microencapsulated iron limit their use, allowing low concentrations, with a perceived metallic taste, in addition to factors such as oxygen, temperature that limit the addition of iron in free form, causing the alterations (Dias et al., 2015).

## 11. Conclusion

The different methods and techniques of iron microencapsulation and its behavior in food fortification have been studied, being the three determining factors for an optimal microencapsulation: the technique used, a correct formulation between the wall material and the core, elements that whose control have managed to have a favorable success rate greater than 73% taking into account the efficiency, yield and bioavailability of the microencapsulates. It has been shown that the wall material has a significant effect on the iron microcapsules, by providing greater stability, better encapsulation structure and useful life, however, at a higher concentration of solids in the material ratio of wall and core, the bioavailability decreases.

## Author contributions

JN-J, HM-M, JV-M, ME-D, ZS-J, LR-F, and LE-E made a concrete, intellectual and scientific contribution to the conception, design and revision of the article, read the final manuscript, analyzed, and approved for publication. 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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