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Current trends in food processing and nutrition to mitigate nutritional health issues

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Current trends in food processing and nutrition to mitigate nutritional health issues

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Editorial: Current trends in food processing and nutrition to mitigate nutritional health issues

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Editorial on the Research Topic
Current trends in food processing and nutrition to mitigate nutritional health issues

This Research Topic was created to promote new studies exploring novel techniques for improving modern dietary choices. innovative approaches to reducing nutrient losses in present methods of food preparation, processing, preservation, extraction, and utilization; integration of functional foods rich in particular bioactive substances, and micro/macro-nutrients with anti-hyperlipidemic, anti-diabetic, anti-inflammatory, anti-carcinogenic, potentials, and so on, are among top priority goals set forward for achieving protected, healthy, and sustainable living. Ten articles (5 review articles and 5 original articles) are published in this Research Topic and a short summary of all these published articles is discussed below:

In a research article, the authors aimed to evaluate the anti-diabetic potential of *Abelmoschus esculentus* (okra) seed extract. Microwave-assisted extraction (MAE) was used to find the best parameters for extracting polyphenolic chemicals from okra seeds. For optimization, a face-center composite design was adopted. The polyphenolic content was investigated while the solvent/dry matter ratio, wavelength, and time were taken into account. Thin layer chromatography (TLC) and Fourier transform infrared (FTIR) spectroscopy were used to characterize the extract obtained under ideal conditions, and it was subsequently examined for anti-oxidant, alpha-amylase inhibitory, and anti-diabetic activity. The best conditions for phenols extraction were determined using response surface methodology (RSM) as microwave power 330 W and a solvent ratio (97.04/1 mL/g) for extraction time of 9.5 min. TLC found that the extract included quercetin and catechin and had a phenolic concentration (86.37 ± 1.13 mg GAE/g). Extract displayed good *in vitro* antioxidant capabilities with 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity and FRAP assay, and functional groups indicative of polyphenols was found on FTIR spectra. The DPPH scavenging test yielded an IC_{50} of 3.99 ± 0.15 g/mL. In an α-amylase inhibitory assay, the optimized okra extract performed as a non-competitive inhibitor of pig pancreatic amylase (IC_{50} of 484.172.33 g/mL). The extract exhibited anti-diabetic efficacy in streptozotocin-induced diabetic male Wistar rats, as measured by food intake, serum lipid profile, fasting blood glucose levels, and changes in body weight, among other things (Woumbo et al.).

A review was conducted to evaluate the parameters impacting sweet potato (SP)'s moisture loss, drying kinetics, serum lipid profile, pre-treatments, conditions for operation, various drying methods, and their efficacy to enhance the functional, and nutritional properties as well as drying process. To acquire concentrated nutrients to enhance energy efficiency while being environmentally friendly, an optimal drying procedure is required. Traditional ways of drying SPs, such as sun or open-air drying stated as long process, which resulted the lower quality. So, different drying methods, such as freeze, infrared and vacuum drying, as well as pre-treatments such as ultrasound and osmotic dehydration, which were widely utilized around the world. The best-fit thin-layer models (Hii, Page, two-term, logarithmic) used for drying SP are also discussed, as are acceptable modeling methodologies for optimizing drying procedures (Rashid et al.).

Another review paper covered bisphenol A (BPA), which is a synthetic chemical that was commonly used in the synthesis of polycarbonate plastics, epoxy resins and polymer materials. BPA is present in the environment, for example, in food containers, toys, medical gadgets, thermal papers, drink bottles, and so on, and is leached into soil/water. It has the ability to change various biological systems because it is a powerful endocrine disruptor. Studies have verified its anti- androgen action and estrogen-like actions, which have a wide range of detrimental health effects, particularly on the neuroendocrine function, immunological system, and reproductive mechanism. According to recent scientific studies, it can also cause mutagenesis and carcinogenesis. The authors concentrated on the presence and amounts of BPA in various settings and dietary sources, as well as the fundamental processes of BPA- induced toxicity and health disturbances. It is a one-of-a-kind review in that it focuses on the link between BPA and cancer, hormone disturbance, and infertility immunosuppression. These problems are widespread today, and BPA plays a substantial role in their occurrence due to its extensive use in everyday utensils and other accessories. The study also examines research-based mitigation strategies for the harmful chemical (Manzoor et al.).

The authors' goal in this study is to review the accomplishments of pulsed electric field (PEF) used to the aging of fermented wine in a systematic manner. According to research on the use of PEF treatment, and PEF in a fermented wine provides the following benefits: (1) reducing the time it takes for brewing materials to macerate; (2) facilitating the extraction process of primary functional compounds; (3) improving the color of fermented wine; (4) deactivating spoilage bacteria; and (5) accelerating the synthesis of scent substances. These are mostly related to changes in molecular structure, PEF-induced bio-membrane electroporation, and the occurrence of chemical reactions. Furthermore, the essential features of PEF treatments after wine fermentation were highlighted, as well as some undesirable effects and future research prospects (Feng et al.).

The authors worked on the potential anti- aging impact of sea cucumber peptides (SCPs) on Caenorhabditis worm model as well as the underlying mechanisms in this study, which used SCPs from *Acaudina leucoprocta*, which were produced using patented bio-enzyme digesting method. SCPs increase the average nematode lifespan by 31.46%. SCPs improve *C. elegans*' anti- stress capacity by boosting motility and heat resistance. Additionally, collected oxidative stress inducers including the reduction of ROS (71.43%) and lipofuscin (40.84%). Furthermore, SCPs can boost nematode antioxidant capacity by increasing SOD and CAT activity and decreasing MDA buildup in nematodes by 32.44% (Wu et al.).

The increasing variety of food processing approaches the ongoing expansion of the food-trade chain, and possibility of threat factors in the process of making food all make people pay a growing amount of devotion to the facility creation, and enhancement of the Hazard Analysis Critical Control Point (HACCP) system. Only endpoint control and afterwards monitoring of food can provide ultimate food safety. It is critical to strictly detect and analyze food safety issues during the processing process. There is a need for the manufacturing companies to establish and implement the HACCP systems, so it is necessary to carry out the primary responsibility of food safety as well as improve the practical application and theoretical understanding of HACCP system in China. The study's objectives were to track the trends and influence of research in this field by Chinese research institutions and significant authors, and to analyze the research. It is crucial for HACCP research to continue. The study's findings revealed that (1) there was a steady increase in HACCP publications in China from 1992 to 2004 before they started to decline; (2) the indexes of journals with more publications were more concentrated, with Food Science publishing the most; and (3) the cultivation bases of the State Key Laboratory of Chinese Medicinal Materials in the Center of Chinese Medicine Resources of China were among the largest research institutions; (4) four additional active research teams have been established in the field of HACCP as a result of the main author indicators. To ensure that food is truly safe, it is advised that China integrate food hazard analysis and assessment into the pre and post-production processes of food (Shi et al.).

In a review study, authors described how residual agro-waste from the pomegranate juice, which was valorized to make pomegranate seed oil (PSO) as well as sunflower oilseed cake was utilized to manufacture the sunflower meal protein concentrate (SMPC). After being removed, both components were mixed to make high nutria omega cookies. A thorough set of laboratory analytical techniques were used to assess the properties of pomegranate seed oil in order to verify its purity and viability. Then, using different concentrations of PSO and SMPC, the HNO5 cookie products were made. The sensory, proximate, physicochemical, and efficacy tests of these cookies were thoroughly examined in order to ascertain their general shelf-life properties and nutritional qualities. The findings showed that 15% SMPC and 15% PSO cookies exhibited stability in a number of sensory tests and physicochemical. Rats' responses to famine were dramatically reduced by the punicic acid in HNO5 cookies, which also gradually enhanced various metabolic functions and general health profiles (Iqbal et al.).

In a trial, the formulated baby meal was made from *Eleusine coracana* (ragi) and *Musa paradisiaca* (Nendran banana). Formulated weaning food was examined using a variety of established techniques, proving that it could give growing infants the nutrients they needed for healthy growth and development. Weaning food's ability to last for 3 months in two different packaging types of aluminum and plastic was also investigated. The

aluminum foil pouch showed the best ability to last. This ready-to-serve food could be viewed as a highly effective supplemental food for infants because it is made with natural ingredients that contain important macronutrients and micronutrients. Additionally, this breakthrough may lead to the introduction of a weaning solution that is specifically designed for poor socioeconomic groups (Kabeer et al.).

The various features of summer savory, including its medicinal qualities, biological activity, food applications, nutritional value, potential health advantages, and use as an addition in grill feed, were covered by the writers of a review study. Additionally, the associated toxicity is covered. Summer savory leaves are rich in total phenolic components, which have strong antioxidant effects. Summer savory contains rosmarinic acid as a primary ingredient. According to phytochemical studies, the main constituents of Satureja species include tannins, gums, phenolic compounds, mucilage, volatile oils, acids, sterols, pyrocatechol, and pyrocatechol. In tests for antioxidant, cytotoxic, and antibacterial effects, summer savory extract demonstrates significant biological potential. Summer savory extract exhibits an inhibitory effect on lipid peroxidation in terms of antioxidant activity. In addition to having minerals and vitamins, summer savory includes Fe (III) reductive and free radical scavenging properties. In addition to having antibacterial and antioxidant characteristics, summer savory also offers preventive effects against cancer, cardiovascular illnesses, Alzheimer's disease, Jurkat T cells, cholesterol, and infections. Due to their antioxidant qualities and high nutritional content, this plant's leaves and stems are used in the pharmaceutical industries and food feed. In conclusion, summer savory is usually regarded as being healthy for people because of its adaptable qualities and medicinal applications (Ejaz et al.).

Spray-dried yogurt powder (SDYP) offers functional qualities that enhance solubility, make other food derivatives such bread and pastries easier to use, process, package, and transport, and have shelf stability. The goal of the current study was to improve SDYP and expand its application in cookie preparation for functional purposes. Different outlet air temperatures (OAT) (65, 70, and 75 C) and input air temperatures (IAT) (150, 155, and 160 C) were used to spray-dry yogurt. However, *S. thermophilus* culture exhibits tolerance to the intense heat approaches, whereas spray drying demonstrates that increasing temperature and increases nutritional loss. The culture of *L. delbrueckii subsp.* Bulgaricus, on the other hand, was discovered to be considerably impacted. The growing concentration of SDYP, baking properties the protein profile and mineral of cookies were all examined for a direct proportional relationship. DPPH antioxidant activity, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and total phenolic content were all considerably impacted. For all characteristics, the sensory profile shows a slope from T0 (0% SDYP) to T3 (10% SDYP), although it starts to slow down as the SDYP concentration rises over 15%. According to this study, a certain temperature combination (OAT: 60°C IAT: 150°C) may be utilized to maximize inoculation culture survival, and this powder can be used to create functional cookies with considerably better sensory and biochemical properties ($P < 0.05$) (Ali et al.).

Author contributions

RA: Conceptualization, Writing—original draft, Writing—review and editing. MT: Writing—review and editing. SY: Writing—review and editing. MR: Writing—review and editing.

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Conflict of interest

MT was employed by Centiv.

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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Energy efficient drying technologies for sweet potatoes: Operating and drying mechanism, quality-related attributes

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Sweet potatoes (SPs) are a versatile tuberous crop used as subsistence and cash crop in raw and processed forms. The major issue with SPs is post-harvest losses, which result in noticeable quality decline because of inappropriate handling, storage, delayed transit, and sales, as well as microbiological and enzymatic activity. Drying is an excellent strategy for managing short postharvest storage life, preserving nutrients, and maximizing long-term benefits. However, several parameters must be considered before drying SPs, such as relative humidity, temperature, drying duration, size, and shape. The current review looks at the factors influencing SPs' moisture loss, drying kinetics, diverse drying methods, pretreatments, operating conditions, and their efficacy in improving the drying process, functional, and nutritional qualities. An optimal drying process is required to preserve SPs to obtain concentrated nutrients and improve energy efficiency to be ecofriendly. Drying sweet potatoes using traditional methods such as sun or open-air drying was found to be a slow process that could result in a lower quality. Various advanced drying techniques, like vacuum, infrared, freeze drying, and pretreatments such as ultrasound and osmotic dehydration, have been developed and are successfully used globally. The best-fit thin-layer models (Hii, Page, two-term, logarithmic) utilized for drying SPs and appropriate modeling methods for optimizing drying procedures are also discussed.

KEYWORDS

sweet potatoes, drying methods, kinetics, mathematical modeling, energy considerations

Introduction

The sweet potato is a tuberous vegetable that is a member of the family Convolvulaceae, cultivated in numerous Asian countries due to its ease of growth and high productivity. The leading producers include China, Indonesia, Nigeria, Uganda, and Vietnam (1). Sweet potato tubers contain macronutrients like starch, fiber, and protein, as well as a variety of micronutrients including minerals (manganese, copper, potassium, and iron), vitamins (primarily B complex, C, and E), and provitamin A (as carotenoids), anthocyanins (purple sweet potatoes), flavonoids, and coumarins (2). When compared to other root and tuber crops, the sweet potato contains more carbohydrates and proteins, as well as certain vitamins and minerals, and it contains more provitamin A, vitamin C, and minerals than wheat or rice (3). Due to its high concentrations of bioactive secondary metabolites, sweet potatoes are gaining popularity among the food industry, consumers, and scientists, not only as a healthy product but also as an ingredient for functional foods. The consumption methods and health-associated benefits of sweet potatoes are listed in Figure 1. It is considered a traditional food crop SPs are classified as perishable if their moisture level exceeds 70% (4). As the higher moisture level makes them particularly predisposed to microbial decay, even when stored at room temperature (5). The postharvest behavior of sweet potatoes is sensitive to storage under ambient and cold temperatures due to sprouting and chilling injuries, respectively. An effective postharvest preservation method such as drying is recommended to expand the shelf life of SPs besides processing them into various by-products. Drying can be performed as a unit operation in agricultural crop processing (6).

Drying is a mass transfer phenomenon, involving the evaporation of water from food. It entails applying heat to a material, which causes internal moisture to be transferred to the surface of the material, which is then removed into the atmosphere (7, 8). It is the conventional and most common way of preserving food, increasing shelf-life and product quality, minimizing the weight and bulk, lowering handling and packaging efforts, as well as freight costs. The airflow promotes heat application by generating and eliminating humidity. The drying process most importantly moisture content, can be easy to be removed when the relative humidity is lower. The dried products retain nutrients, color, and fragrance if the drying process is appropriately controlled. The efficient drying of fruits and vegetables allows consumers to enjoy these delicacies even during the off-season.

Food drying includes traditional (sun or wind-drying), and non-traditional methods (infrared, vacuum drying, microwave, and freeze drying). Modern drying techniques allow uniform drying in a shorter period and extended storage life of up to a year or more. Recent advancements have produced drying processes that focus on improving existing methods to deliver high-quality goods using less energy, such as freeze-drying, vacuum, microwave, and microwave-combined freeze-drying (9). Refractance window drying is a popular method for converting liquid food and other biomaterials into high-value powders, flakes, or sheets. This drying technique is simple and affordable for freeze-drying, but it requires large installations to be cost-effective (10). Finally, microwave drying can reduce drying time and thus enhance the quality of the final product (11).

There are many studies on sweet potato drying, however, they lack collective information that would help researchers who want to carry out more research. Similarly, different factors must be considered, such as drying methods, pretreatments, kinetics, nutrient degradation, energy activation, and mathematical modeling. The myriad factors make it difficult for researchers and concerned food industries to understand the pros and cons of the technologies to adapt an appropriate method for drying SPs. This study aims to provide a comprehensive review of the influence of numerous drying techniques on the water loss ratio of SPs and simplify the drying kinetics and other necessary operating conditions to achieve quality and nutrient preservation in the final product. The key objectives are to explore the impact of operating conditions in drying SPs, appropriate drying methods, osmotic and chemical pretreatments, effects of drying processes, nutritional qualities, models that are most suited for drying, energy consumption, and further improvement with the recent trends in SPs drying techniques.

Sweet potato drying approach

The drying process begins with removing unbound surface moisture and progresses through the removal of bonded humidity inside the food material until a predefined limit is achieved. Mass and heat are transferred at the same time in the drying. Agricultural commodities require a favorable drying environment, high temperature, and a high capacity of air to absorb water or low relative humidity during the operation.

The drying process of sweet potatoes product comprises four stages after a preheating period, as depicted in Figure 2A. Stage I, with a constant drying rate, refers to the frame of energy absorption for the moisture release, followed by a stage of evaporation at a decreasing rate. Both are key factors influencing the complete drying mechanism (12). During stage II, evaporation occurs from the food material's external surface to remove physically attached moisture (free water) at a fixed drying rate. This phase did not observe during sweet potato drying due to an insignificant amount of unbound moisture in SPs (Figure 2B). For SPs, stages III and IV show a declining drying rate until the anticipated moisture content is attained. When the transportation of moisture from the inner of the

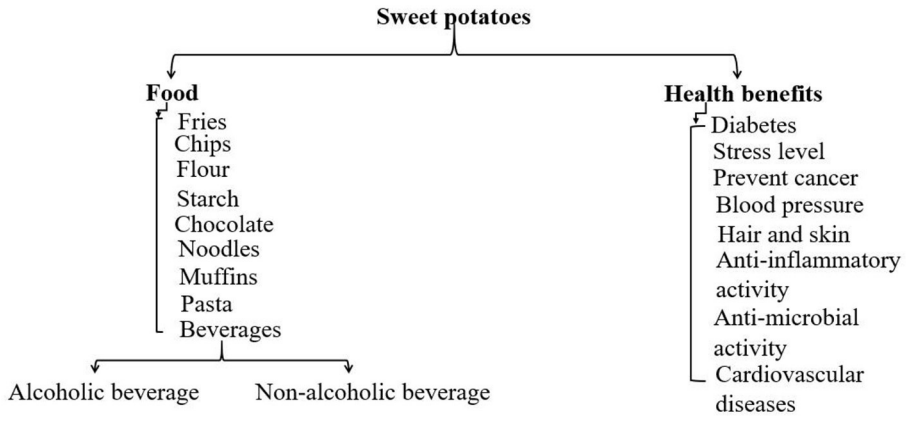

FIGURE 1
Classification of and health benefits of dried sweet potato products.

material to the outer surface (low concentration gradient) slows down (as compared to the evaporation at previous stages at a constant rate) and results in a decline in drying rate. When the equilibrium moisture content is achieved, no further moisture exchange occurs between the material and adjacent air, which is the end of the drying process. Dehumidified air at high temperatures can be used to continue drying SPs below the equilibrium moisture content.

The role of operational conditions in drying sweet potatoes

Improper drying often harms the quality and nutritional profile of a food item. Therefore, optimal drying conditions are necessary to produce prime-quality dried SPs with their optimum nutritive and functional potential. To optimize the drying method for SPs, certain factors must be considered, including slice thickness, drying time, and climate conditions. Furthermore, to get the anticipated moisture level for the food materials, the relative humidity under isothermal conditions can be appropriately controlled by drying techniques. Some industrial dryers suitable for drying SPs are listed in Tables 1–4, along with their effects on the drying process' effectiveness.

Relative humidity

Relative humidity (RH) is the ratio of saturated to humid air's water vapor pressure. It is an important drying parameter that affects the transport of mass and heat as well as the quality and efficiency of drying. It shows the degree of divergence from saturated moist air and the moisture absorption ability of the drying medium moist air. However, the mechanism by which RH influences drying behavior is unclear. As a result, there is no definitive RH control strategy for drying fruits and vegetables. It was found that continuous dehumidification during drying may increase drying efficiency, and the lower the RH level, the faster the drying rate, as observed in rapeseed and spaghetti (59, 60). Only a few studies have considered the effect of RH in sweet potato drying, thus optimizing RH is still necessary in this case. According to Sun et al. (19), the elevated drying temperature may reduce the RH of the drying medium and increase the vapor pressure shortfall, boosting the degree of external mass transfer. Similarly, Sabudin et al. (61) discovered that 40% relative humidity had the highest drying rates and moisture gradient in sweet potato drying.

Air velocity

The uniform air circulation defines the drying operation duration and the final dried product quality. Generally, hot air maintains a specified temperature range (40–50°C) in the dryer throughout the drying process. The surrounding fresh air absorbs the moisture from the product's outer surface with low RH%, which increases moisture removal efficiency. The airflow over the product's surface ensures rapid moisture removal by evaporation and the maximum drying rate at a constant air temperature and changing ambient RH. Fast air circulation has a high potential to replace the stationary boundary layer of drying air from the surroundings of a humid product. SPs might exhibit a similar phenomenon under higher air velocity. A high drying rate is not usually suggested since it might harm the food matrix, causing cracking or deformation. Laminar airflow might be observed when using a low-speed fan, allowing air passage into the stack, leading to ineffective distribution and lower heat exchange between the air stream and product surface. Walker (62) and Zhu and Jiang (17) noted that the drying operation of

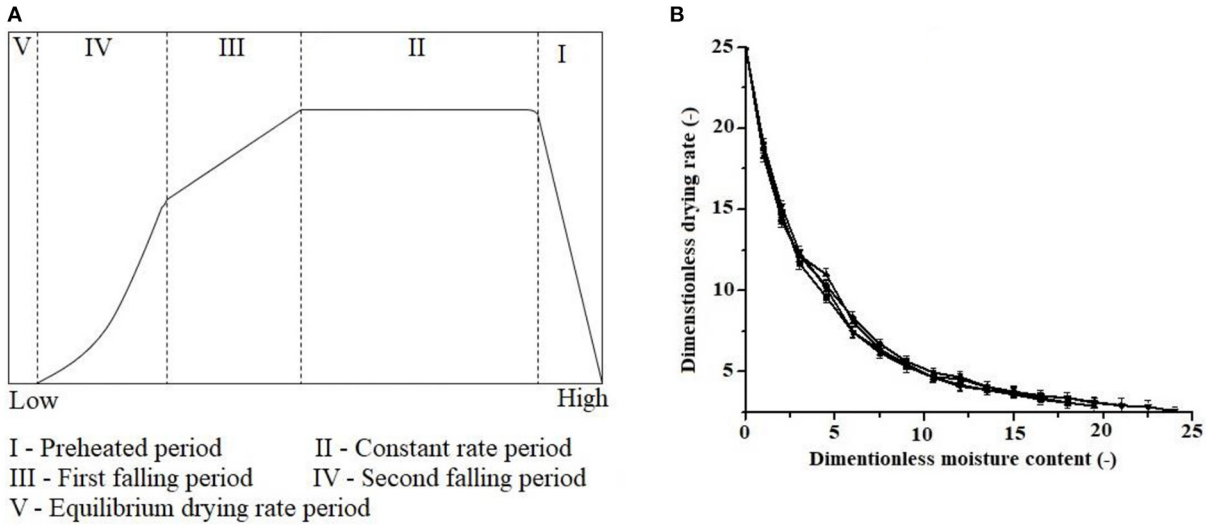

FIGURE 2
(A) Typical stages of food product drying, **(B)** drying curve of sweet potatoes.

SPs is highly influenced by air velocity. In the Wang and Singh model, air velocity and slice thickness are substantial (18).

Drying time

Drying time and air temperature are inversely related; lowering drying time requires increasing the drying air temperature. The heat transfer increases in direct proportion to the moisture gradient, speeding up the drying. It is attained by the amount of heat provided to the material and moist air is removed from the surrounding area. The drying operation of drying is mainly propelled by the moisture gradient. A higher gradient along the equilibrium might result in faster drying. Otherwise, the absence of a sufficient gradient can cause a reduction in the drying rate and, consequently, may extend the drying period. Significant quality degradation, color deterioration, and structural changes can occur because of prolonging the drying time. Sample thickness, wither degree, temperature, and air velocity are critical parameters for calculating the time needed to produce dried SPs. The continuous moisture removal decreases the moisture content as the drying operation proceeds (13). Using de-humidified drying air and low temperature can enhance the degree of drying and reduce the drying period capture oxidation. During this drying period, the rate of evaporation increases significantly. Retaining the quality and flavor to a high degree is a promising outcome for the obtained product. Furthermore, the product has a longer shelf life and might be used after a long time without degradation.

Thickness of slices

The size of slices, especially the thickness, is an equally important parameter in the drying operation. A reasonable depth of sweet potato slices is critical to circulate drying air through the voidage from channels, particularly in a fixed bed, conventional, low-capacity tray batch dryer. Thin packable layers are commonly employed to disperse food ingredients. The thick loose layers need for large food items. These materials can induce airflow resistance and may require slightly more time for drying. The transportation of the internal moisture becomes extremely tough in thick slices, owing to decreased mobility in the food matrix, resulting in a lower moisture removal rate. Producing dried SPs with the desired moisture content of <7% requires a very long drying process and often generates uneven products because of insufficient contact between incoming hot air and sweet potato slices (15). Overloading can cause a longer drying time with a need for an expansion in air temperature. The degree of wither and inlet temperature significantly influence the calculation of the thickness and pitch of the spread. Drying time may be reduced, and low-frequency agitation applied to the drier bed can enhance the quality of dried SPs.

Rehydration

Rehydration is a complicated procedure that aims to restore the raw product's properties. Rehydration capacity is influenced by drying processes and other parameters such as rehydration duration, product composition, and water temperature. A key step in producing dried foods is quick and thorough

TABLE 1 Hot air-drying (HAD) studies were conducted to dry sweet potatoes.

Drying conditions/Temperature (°C)/Geometry	Size (mm)	Response	Main conclusion	References
HAD; T = 50–90°C; V = 1.0 m/s; RH = 50%; G = Slices	3–8	Thin layer models, D_{eff}, E_a	Hii model showed results for D_{eff} (3.66 10^{-10} to 2.11 10^{-9} m^2/s) and E_a (13.48–16.40 KJ/mol)	(13)
CD; T = 50–90°C; V = 1.5 to 5.5 m/s; RH = 23 and 50%; G = Cubes	5–12	Thin layer models, D_{eff}, E_a	E_a was 11.38 KJ/mol; D_{eff} increased with temperature. Page Model performed better	(14)
TD and FBD at 70 and 80°C; G = Cylinders	–	Drying, blanching, shrinkage, color	Fluidized bed dryer temperature didn't affect shrinkage, quality and appearance were better, and blanching improved color	(15)
CFD = 42°C, SD = 27–50°C; G = Slices	–	Provitamin A carotenoid analysis, moisture/water activity	Solar and sun drying retained Provitamins A. Vitamin A was rich in orange sweet potato flour	(16)
Tunnel drying; T = 60–80°C; V = 0.42 to 1.12 m/s; RH = 10–15%; G = Slices	–	Mathematical modeling, E_a	The logarithmic model best fits drying data. E_a = 23.29 KJ/mol	(17)
OD; T = 50–80°C; G = Slices	5, 10, 15	Mathematical modeling, E_a	Page model and modified page model best described drying. E_a = 11.10–30.40 KJ/mol	(18)
HAD; T = 40°C, V = 1 m/s; G = Slices	5	Hyperspectral imaging, modeling	Hyperspectral imaging was a fast way to predict moisture content, and the RC-MLR model was best	(19)
HAD; T = 50–90°C; G = Slices.	5, 8, 12	Neutral network, MC, drying kinetics	ANN models can be used to estimate drying online	(20)
DD; T = 120, 130 and 140°C; G = Slices	–	Phytochemicals and antioxidants	Higher TPC and antioxidant activities at 140°C and drum-dried flour have higher antioxidants	(21)
CD; T = 50, 60, and 70°C; G = Slices	30 × 48 × 5	Drying kinetics, rehydration ratio	The logarithmic model best fit with E_a at 23.2 KJ/mol	(22)
TD; T = 50–80°C; V = 2.5 m/s; RH = 10%; G = Slices	–	Drying kinetics, blanching	Page model fits best, and blanched samples dried faster than unblanched	(23)

HAD, hot air dryer/drying; CD, cabinet dryer; TD, tray dryer/drying; OD, oven dryer/drying; DD, drum dryer/drying; ANN, neutral network approaches; US, ultrasound; D_{eff}, moisture effective diffusion/moisture diffusivity; E_a, activation energy; SEM, scanning electron microscopy; TPC, total phenolic content.

rehydration. Moreover, drying conditions, pretreatments, and structural properties of dried products can significantly affect the rehydration capacity (63). Water content slowly decreases during the drying process, resulting in irreversible cell damage and displacement, loss of cell integrity, structural collapse, and loss of hydrophilicity. The rehydration index can be determined using Equation (1):

$$R = \left(\frac{W_d}{W_r}\right) \times 100, \quad (1)$$

W_d and W_r are the dehydrated and rehydrated sample masses (g).

As drying temperature increases, the rehydration index increases as well. The contraction causes the sweet potato's cellular structure to be very porous, enabling more water absorption. SPs demonstrated the least water adsorption by drying at 40°C, while those at 80°C demonstrated the quickest adsorption, perhaps because of decreased shrinkage (63). According to Doymaz (64), SPs have a high moisture content. As a result, water molecules enter SPs with relatively little driving force.

Classification of drying methods for sweet potatoes

SPs can be dried in various ways, as seen in Figure 3. Table 1 contains descriptions of the drying processes.

Fluidized bed drying

Wet particle drying is accomplished by utilizing FB dryers. Additionally, suspensions, granular materials, pastes, and slurries, can also be fluidized in inert solids bed. FB dryers improve the efficiency of high heat and mass transfer, uniform moisture reduction, good solid mixing, and accessible material transportation (65). They maintain a consistent bed temperature throughout the drying process and extend the constant drying

TABLE 2 Hot-air drying combined with other drying methods was conducted to dry sweet potatoes.

Drying methods	Drying conditions/Temperature (°C)/Geometry	Size (mm)	Response	Main conclusion	References
Air/sun/solar drying	HAD, OSD, and STD; G = Slices.	–	Provitamin A, carotenoids contents	OSD and STD didn't affect carotenoids. Low-temperature storage decreased provitamin A	(16)
Air drying/freeze drying	OD = 30, 70 and 100°C; FD = −20°C, G = leaves; V = 1.5 m/s	–	CQA derivatives and antioxidants	Freeze-drying preserves CQA and antioxidants, while 70 and 100°C drying preserve both of them	(24)
Air drying/microwave/ vacuum	OD = 65°C, 9 h, P = (800 W, 50 Hz), 5 min, FD = −50°C, 36 h; G = Slices	5	Antioxidants, phenols, ascorbic acid	Microwave drying increased TPC and antioxidants. β-carotene reduced sweet potato slice's antioxidant activity.	(25)
Spray drying	Inlet T = 200°C; Outlet T = 100°C; G = Puree	–	Physicochemical, antioxidants	MD flour had a high antioxidant and retained anthocyanins, total phenols, and flavonoids.	(26)
Sun drying	OSD; T = 54°C; T = Slices	1.5	Protein content, fiber, moisture, β-carotene	Matobolwa sweet potato retained more β-carotene than michembe variety after 6 months' storage	(27)
Air/Sun/Solar tunnel drying	T= 57°C, STD T = 45–63°C, OSD T = 30–52°C	1–2	Carotenoid profile, β-carotene, color	Forced-air oven drying, STD, and OSD reduced all-trans β-carotene by 12, 9 and 16%	(28)
Air drying/IR radiations	HAD T= 50°C, 55 and 60°C, IR T = 250 W, HP T = 180°C; G = chips	6, 8	Drying kinetics, sugar, taste, texture	IR radiations assisted thick sweet potatoes at 60°C. IR radiation at 60°C for 5 h and HP drying was suitable for mass-producing sweet potato snacks	(29)
Spray drying	T = 85°C; G = slices	2-3	Phytochemicals, antioxidants, color, microstructure	Encapsulated flour had higher TPC, antioxidant, and water solubility than non-encapsulated flour	(30)
Intermittent IR and convective drying	IR= 1,100 and 1,400 W/m^2; G = slices	4, 36	Mathematical simulations, color, microstructure	IR drying is more effective than convective hot air drying for product quality	(31)
Air/Microwave-vacuum drying.	SBD, HAD = 80°C; MVP = 2.0 W/g; VP = 5 kPa; G = dices	10 × 10 ×10	MC, rehydration ratio, crisp degree, expansion ratio	Microwave-spouted bed and MVP showed faster drying, better rehydration, uniform color, and high β-carotene retention than HAD	(32)
IR drying	P = 104, 125, 146, 167 W; G = slices	3, 5, 8	Drying kinetics, rehydration ratio	Increasing power reduced drying time. IR affects RR and D_{eff}. The log model fits drying curves	(33)
Air drying/IR/Combine IR and air drying	HAD = 50, 60 and 70°C; IR = 1,100, 1,400 (W/m^2); G = slices	4, 5	Drying kinetics, energy consumption, E_a, color, microstructure	Combined IR and HAD drying had the shortest drying time, lowest energy consumption, and best color attributes	(34)

(Continued)

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TABLE 2 (Continued)

Drying methods	Drying conditions/Temperature (°C)/Geometry	Size (mm)	Response	Main conclusion	References
Sun/Tent/tunnel drying	T = 10, 20, 30, 40°C; G = slices	2	Carotenoids, dry matter, drying kinetics	OSD decreased carotenoids less than tent and tunnel drying	(35)
Air/tray/infrared drying	FBD = 45, 55, 65°C; tray = 3,000 W; IR; 1,000 W; G = pieces	–	Mathematical modeling, starch properties	Midilli model best explains starch drying. The drying affected starch's color, solubility, and gel texture	(36)
HAD/IR drying	HAD = 50, 60, 70; V = 1.16 m/s; RH = 45°C; IR = 1,100, 1,400 W/m$^{2;}$ G = Slices	4–6	Mathematical modeling, E_a, color	The two-term model explains sweet potato drying kinetics. E_a = 12.83 to 34.64 KJ/mol	(37)
IR-hot air drying	IR-HAD = 60°C; G = slices	4	Heat and mass transfer	Lambert's law explains sweet potato heat and mass transfer	(38)
IR heating	IR = 1,400 W/m^2; G = slices	4	Heat and mass transfer, shrinkage	Mass and heat transfer coefficients, shrinkage, and IR heating affected moisture distribution during drying	(31)
Tunnel/shade/open-air drying	G = chips	–	Carotenoid contents	All dryers lost 9.2% carotenoid. After 4 months of storage, the carotenoid loss was 83%	(39)
Sun drying	OSD, 10 a.m. to 4 p.m.; G = leaves	–	Proximate analysis, anti-nutrient content	Purple midrib sweet potato leaves had more fiber, ash, carotenoids, iron, calcium, and polyphenols	(40)
Microwave drying	MSBD = 60°C; Steamed = 100°C; G = Cubes	10 ×10	Rehydration, color, texture, anthocyanins	Steamed coating with sodium alginate reduced drying time, improved color, and reduced rehydration	(41)
Vacuum drying	VD = 100°C, 120°C, and 140°C; P = 2.67 kPa; G = Chips	0.80, 1.50	Drying kinetics, β-carotene, color, texture	High-likeability mix temperatures maintain color and -carotene	(42)
IR/Air drying	IR = 1,100 W/m^2, HAD = 50–70°C; G = Slices	4	Drying, phytochemicals, color	The two-term model fits best, and IR-HAD was the best method	(43)

HAD, hot air dryer/drying; OSD, open sun drying; STD, solar tunnel dryer/drying; OD, oven dryer/drying; FD, freeze drying; IR, infrared; MVP, microwave-vacuum drying.

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TABLE 3 Application of different pretreatments along with varying methods of drying on drying of sweet potatoes.

Drying methods	Drying conditions/ Temperatures (°C)/Geometry	Pretreatments	Size (mm)	Response	Main conclusion	References
Catalytic infrared drying	IR T = 60, 70, and 80°C; G = Slices	Multi-frequency US drying (20, 40 and 60 kHz)	3	FTIR, SEM, phytochemicals, drying kinetics	40 kHz at 70°C reduced drying time. Ellagic acid and Rutin were higher, and 20 kHz. FTIR showed the OH group and phenolics	(44)
Microwave drying	P = 700-W; G = Circular slices	Carbonic maceration (CM)	5	Drying, physicochemical, and antioxidant properties	Intermittently dried CM has high phytochemical and antioxidant activity, reduces drying time, and lowers E_a (DPPH)	(45)
Freeze drying	FD = 13.3 Pa; G = Cubes	Heat treatment after high-pressure treatment	20 × 20 × 10	Rehydration, color, rheological properties	Pretreatments did not affect color or gelatinization rate but improved texture	(46)
HAD	HAD+US; T = 40, 50, 60, 70°C; G = Slices	Drying kinetics, moisture diffusion, energy consumption	5	Ratio and reduce energy consumption	US power increases reduce drying time, color difference, and rehydration	(47)
Contact US-HAD at 40°C	US-HAD; T = 40°C; G = Slices	Hyperspectral imaging, anthocyanins	5	–	RC-MLR predicts anthocyanins best	(48)
Natural drying	OSD, STD; G = Slices	Blanching with boiling water	1.50	β-Carotene, mineral content	Fresh samples had less-Carotene and more fat, protein, fiber, and carbohydrates	(27)
Air drying	HAD; T = 50–80°C; G = Slices	Boiled water blanching, slices	5, 10, 15	Drying kinetics, mathematical modeling, E_a	Page and modified were the best models; E_a was 11.1–30.4 KJ/mol	(18)
Ai drying	HAD; T = 50–80°C; V = 1.25 m/s; G = Slices	Boiled water and metabisulphite blanching, slices	4	Drying kinetics, mathematical modeling	The modified page model was the best. Boiling water and metabisulphite improved drying over control	(49)
Air drying	HAD; T = 55–65°C; G = Flour	Sodium hydrogen sulfite ($NaHSO_3$)	1	Phytochemicals, color, SEM	Pretreated flour showed higher phytochemicals and color change than control	(50)
Air drying	HAD; T = 55–65°C; G = slices	Citric acid pretreatment	1	β-Carotene, ascorbic acid, TPC	β-carotene, TPC, and ascorbic acid values were close to predictions	(51)
Sun drying	OSD; G = Slices, chips	Sodium metabisulphite, ascorbic acid, citric acid, and salt	–	Total carotenoids, storage of sweet potatoes	Ascorbic acid, sodium metabisulphite, citric acid, and salt improved carotenoids in the first month of storage but not after 4–6 months	(52)

HAD, hot air dryer/drying; CD, cabinet dryer; TD, tray dryer/drying; OD, oven dryer/drying; DD, drum dryer/drying; US, ultrasound; D_{eff}, moisture effective diffusion/moisture diffusivity; E_a, activation energy; SEM, scanning electron microscopy; TPC, total phenolic content; KMS, potassium metabisulfite; FTIR, Fourier transform infrared spectroscopy; POD, peroxidase enzyme.

rate period. However, due to high moisture variation, stratified flow and hotspot formation in FB dryers can cause product damage and quality loss. Special additives are required to handle materials and sticky hygroscopic products in an FB

TABLE 4 Application of different osmotic solutions as pretreatments along with varying methods of drying for optimizing drying protocols for sweet potatoes.

Drying methods	Drying conditions/ Temperature (°C)/Geometry	Osmotic solution	Size (mm)	Response	Main conclusion	References
Air drying	HAD; $T = 30–60°C$; $G =$ Slices	Sugar solution 40, 50, and 60%	3, 5	Water loss (WL), solid gain (SG)	At 60°C, a 60% sugar solution had the best WL and gain. WL and SG were highest in controls.	(53)
Air drying	$T = 60°C$; OD = 10–15% w/v; $G =$ Slices	NaCl solution	2,3,4	β-carotene, D_{eff}	Salt did not affect β-carotene degradation, but OD decreased.	(54)
Air drying	OD = 0, 5, 10% w/w; G = Slices	Sucrose, sorbitol, fructose	20 × 20 × 5	WL, SG, water activity	Sorbitol's WL was higher than fructose's SG. Barbosa Junior showed the best fit model	(55)
Air drying	OD = 40% sucrose, 5% w/w salt; VD = 70°C; G = Slices	Sucrose, NaCl	0.5	WL, SG	Azuara model best fit. WL and SG of sweet potatoes increased with time	(56)
Ultrasound microwave vacuum drying (MVD)	HAD; $T = 65°C$; OD = 35°Bx; MVP = 22 W/g; P = 90 KPa; $G =$ Slices	Sucrose solution	10	WL, SG, expansion ratio, rehydration ratio, color, texture, SEM	The US rehydrated more. The osmotic group improved WL, and SG improved color, taste, and texture. Osmotic and US drying improved	(57)
Microwave drying	$P = 180$ and 350 W; OD = 1% w/v; $G =$ Slices	Sucrose, fructose, sorbitol	20	Drying kinetics, mathematical modeling	Weibull fits best. OD and microwave power reduce drying time	(58)

HAD, hot air dryer/drying; CD, cabinet dryer; TD, tray dryer/drying; OD, oven dryer/drying; DD, drum dryer/drying; US, ultrasound; D_{eff}, moisture effective diffusion/moisture diffusivity; E_a, activation energy; SEM, scanning electron microscopy; TPC, total phenolic content; WL, water loss; SG, solid gain; HTST, high-temperature short time.

dryer. However, a significant drawback of FBs is the chance of a decline in particle size reduction owing to collision and attrition (66). FBD is one of the most effective drying techniques when solely considering thermal efficiency (67). It is the most effective drying technique for granulated materials because its mixing features promote intense mass and heat transference, resulting in a short drying period (36). Hatamipour et al. (15) explored the drying characteristics and kinetics of six sweet potato varieties and concluded that FBD produces excellent quality and nutritional values for all types. Similarly, a study conducted by (36) on SPs showed that the effective diffusion rate (D_{eff}) in dry beds from 45 to 65°C was 4.92×10^{-7}-7.26×10^{-7} m^2/s, which was considerably greater than infrared and tray drying (Table 1). Similarly, the activation energy (E_a) is almost twice in FBD (17.33 kJ/mol). Because of the high heat and mass exchange rates, FBD has a rapid drying rate. High heating rate is produced with tightly controlled temperature in the bed; when infrared (IR) was combined with the FBD, the drying rate and quality of the SPs were significantly improved (68). Continuous high-capacity FBD technology is extensively utilized in the pharmaceutical, food, fertilizer, and many other chemical industries.

The potency of using fluidized bed dryers is greatly influenced by energy efficiency. This is expressed as the amount of energy applied to vaporize water from solids (E_w) to power provided to the air during drying (E_a), with the energy consumed to evaporate moisture calculated as:

$$E_w = M_w \times L_w \tag{2}$$

E_w = evaporated water energy (kJ),
M_w = water mass evaporated (kg),
L_w= latent heat water vaporization (kJ/kg).

The energy required to heat the air can be calculated as follows:

$$E_a = M_a \times C_p \times \Delta T \tag{3}$$

Where,
E_a = the amount of energy used to heat the air (kJ),
M_a = mass of air (kg),
C_p = the air's specific heat (kJ/kg°C),
Δ_T = temperature difference (°C).

As a result, the thermal dryer efficiency, η_{th}, can be calculated as follows:

$$\eta_{th} = \frac{E_w}{E_a} \times 100\% \tag{4}$$

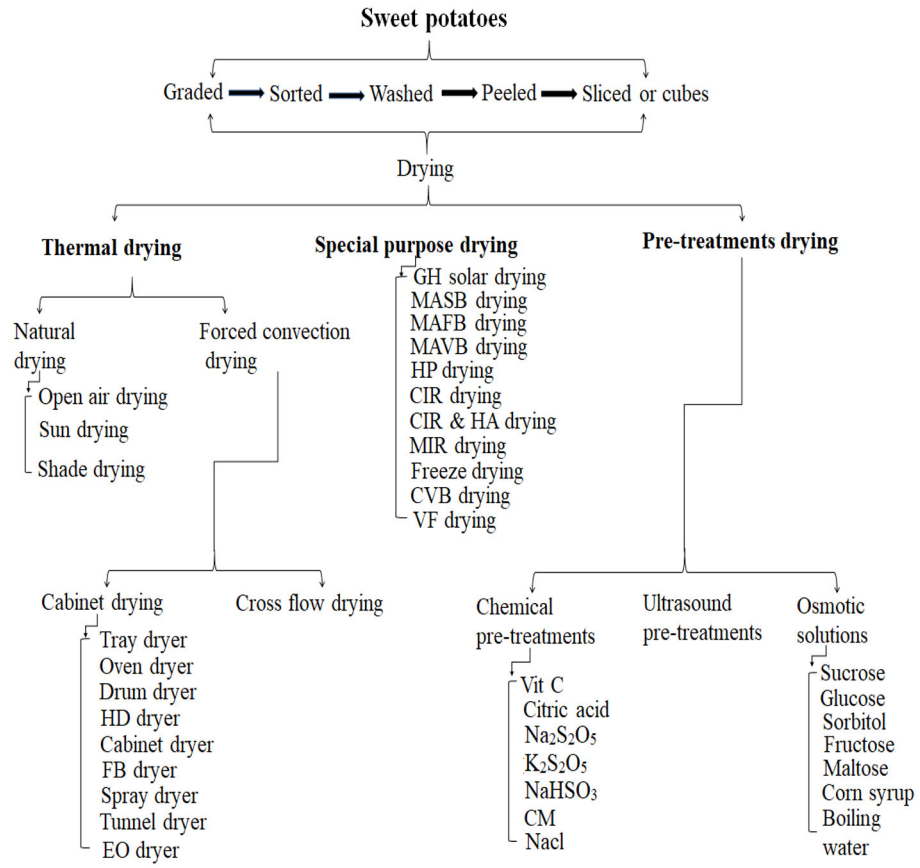

FIGURE 3
Classification of different drying methods used for sweet potatoes.

Oven drying

Oven drying (OD) is a simple method for drying food because it does not require any special or extra equipment (Table 1). Its drying rate is faster than that of sun-drying (Figure 4A) and green house dryer (Figure 4B). However, a significant disadvantage is that it can only be done on a limited scale. Since thermal ovens generally feature chamber volumes ranging from 20 to 800 L with a 5–300°C temperature range above ambient temperature. Heat is transmitted to the chamber through the air flow. Antonio et al. (55) reported higher water loss in SPs slices with NaCl and sucrose solution.

Similarly, adding NaCl to the permeable solution increased the driving force of the process, and the dehydration process was verified to be mainly affected by changes in the NaCl concentration. Clifford et al. (54) found similar results: NaCl solution reduced significant water loss in yellow flesh SPs (YFSP), but the salt solution had no significant effect on β-carotene reduction, although degraded β-carotene. Bengtsson et al. (28) studied oven-dried orange-SPs (var. Ejumula) and observed an 11 and 16% loss of β-carotene content during oven drying at 57°C.

The total energy was estimated by Beigi (69) in oven drying using the following equation is:

$$E_t = E_{th} + E_{mec} \tag{5}$$

Where E_{th} = sum of thermal energy,
E_{mec} = sum of mechanical energy,

$$E_{th} = (A.v.\rho_a.Ca\Delta T).t \tag{6}$$

Where A, v, Δ *and* T are the area of the tray,
(m^2), airflow rate (m/s), and disparity in temperature (K), respectively.

Also, ρ_a = density (kg/m^3) and C_a = specific heat capacity (kJ/kg/K) of inlet air.

$$E_{mec} = \Delta\rho.m_{air}.t \tag{7}$$

ΔP = pressure difference (mbar) and m_{air} =inlet air mass (kg).

Cabinet drying

Cabinet dryers are the most used in the food industry, as shown in Figure 4C and listed in Table 1. In contrast to oven dryers, which usually increase the temperature of the product to allow preheating or curing, cabinet dryers subject the SPs to 50–80°C temperature for 2.5–24 h. Alternatives to such dryers are tunnel dryers or biomass dryers using charcoal or firewood, which also require fuel investments that lead to poor product quality unless produced sustainably. Despite the drawbacks of cabinet dryers, several studies have been done on cabinet-dried SPs' drying kinetics and nutritional aspects. Kosambo (70) used an electric cabinet drier to dry. Drying fresh slices of 13 orange flesh sweet potatoes (OFSP) cultivars at 58°C for 4 h, resulting in a 35% drop in trans-carotene content. Whereas drying was performed by using cabinet drying and open-air sun drying, losses for SPK004 were found to be 28 and 83%, respectively, and 47 and 72% for Jonathan kinds of SPs samples, respectively. As a result, cabinet drying retained more provitamin A than sun drying.

Similarly, Lohachoompol et al. (71) investigated the effect of cabinet drying on the anthocyanin content of blueberries (*Vaccinium corymbosum* L.) and discovered a 49% loss after drying. However, Joykumar Singh and Pandey (72) studied a different aspect of cabinet drying and observed that the effective moisture diffusivity augmented with rising temperature. Higher forced convection drying reduces heat loss and thus, enhances the dried SPs quality (Figure 4D). The fan or blower propels the drying medium (typically air) through the heater to increase the temperature and lower the RH of the air, thereby improving the heat and mass transfer rate (73). SPs dried at a decreasing rate with an inconstant drying rate during forced convection drying. SPs dried in forced air retained more β-carotene than those dried in open-air (28). To achieve the final moisture level, the drying time of the forced convection tray dryer was maintained at half of that of the free convection dryer by (15). The logarithmic model best fit for the drying data (17). Similarly, Doymaz (64) reported a logarithmic model indicating the best-fit model with the shortest drying time in blanched SPs slices.

The energy efficiency was considered as the ratio of energy used to energy input as below:

$$\eta_E = \frac{E_i - E_o}{E_i} = \frac{m_a(h_{dci@T} - h_{dco@T})}{m_a h_{dci@T}} \times 100 \qquad (8)$$

Where η_E Is the energy efficiency%, m_a is the mass of air (kg s^{-1}); E_i and E_o are the input and output energies in kJ s^{-1}. $h_{dci@T}$ is the enthalpy of air at the drying chamber's inlet at temperature. $h_{dco@T}$ is the air enthalpy at the drying chamber's outlet at temperature T.

Specially modified dryers

Microwave spouted/assisted bed drying (MSBD)/(MASBD)

More consistent drying can be achieved with a microwave-enhanced spouted bed. Pneumatic agitation in spouted bed dryers allows items to be exposed to microwave energy uniformly (32). Fluidization also enhances mass and heat exchanges because of a constantly replenished boundary layer at the particle surface. As a result, a combined fluidized-spouted bed is an efficient method for resolving the irregular problems of MW drying. These dryers are more effective than standard dryers because of their shorter drying time and better jet velocity to guarantee excellent mixing (32). They have been widely employed in various industrial processes (Table 2). The continual movement of sweet potato cubes within the microwave chamber accounts for the rapid drying rate in MSBD (Figure 5F). Furthermore, the constant movement of the cubes allows various areas of the system to receive relatively homogeneous MW radiation. MSBD-dried sweet potato cubes absorb microwave energy more equally than fluidized samples. Purple flesh sweet potatoes (PFSP) subjected to be dried in a microwave-assisted spouted bed drier (MWSP) had a low rehydration capacity due to the amylose to amylopectin ratio, which is not suited for microwave heating. Due to the rapid movement of products with higher microwave power, products were crispier than those from other driers. A greater microwave power causes fast moisture evaporation during the MSBD process, which causes the formation of porous structures (32). Although steam blanching can help keep color and anthocyanin content, MASBD drying PFSP cubes is generally not an appropriate processing technique, even with the coating treatment (41).

The drying process's energy efficiency (η_e) is described by,

$$\eta_e = \frac{W_d[h_{fg}(M_{p1} - M_{p2}) + C_m(T_{m2} - T_{m1}) + m_{fw}\left(h_{fw2} - h_{fw1}\right)]}{m_{da}(h_1 - h_o)\Delta t + \Delta t Q_{MW}} \qquad (9)$$

Where W_d weight of dry material (kg); c_m = material specific heat (kJ/kg K); h_{fg} = is the latent heat of vaporization, M_p = dry based particle moisture level (kg $_{water}$/kg $_{solid}$); P_1 microwave power density immersed by a dielectric material (kW/cm^3); P_2 energy is needed to heat material (kW); Q_{MW} =microwave energy (kW).

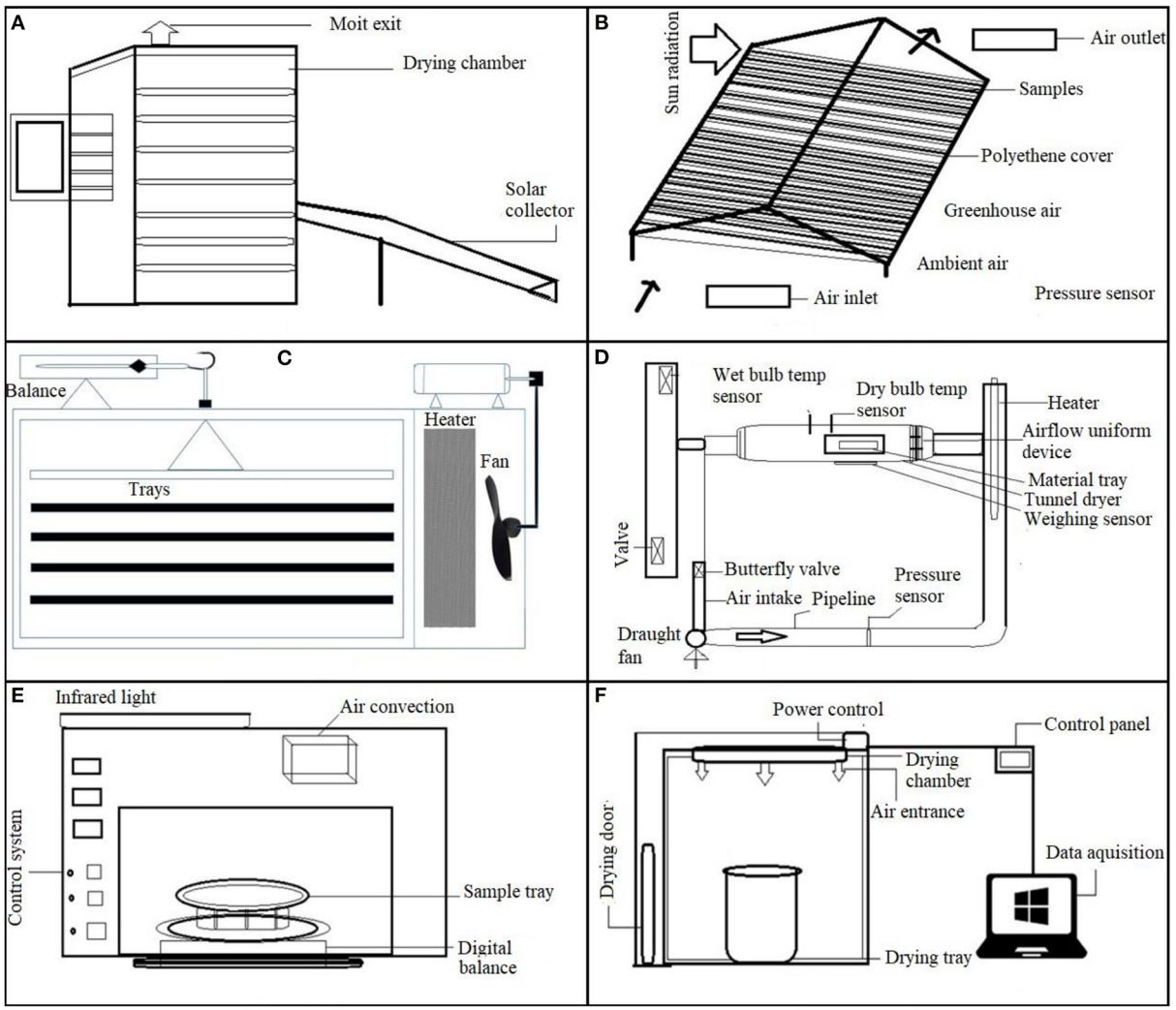

FIGURE 4
Drying techniques used for sweet potatoes. **(A)** Indirect solar dryer. **(B)** Greenhouse dryer. **(C)** Cabinet dryer. **(D)** Drying system. **(E)** Infrared drying equipment. **(F)** Mid-infrared dryer.

Microwave-assisted freeze-drying

Microwave heating replaces traditional conduction heating during freeze-drying, allowing the benefits of both microwave drying and freeze-drying to be combined in a process known as microwave-freeze drying. The combined freeze-drying and microwave energy use is microwave-assisted freeze-drying (MWFD) (Table 2). The microwave drying system adjusts the control sample temperature, sample mass, and microwave power (74). Huang et al. (75) revealed that MWFD chips had the best quality, were preferred by customers, and had a shorter drying time than FD chips in restructured mixed potato with apple chips. In addition, microwave energy increases the drying rate, final product quality, and energy consumption. A study by Liu et al. (41) demonstrated that MWFD is a time-consuming process for drying SPs, and energy consumption was twice as high as in microwave vacuum drying (MWVD) (Figure 5C). However, MWFD could preserve better anthocyanins, and sensory evaluation was remarkable concerning the crispiness of SPs. The potential of this drying technique requires further exploration of SPs.

An electric energy meter can calculate the energy consumption of the three parts during drying: the vacuum system, cold trap, and heating system.

$$ES = \frac{E}{M} \tag{10}$$

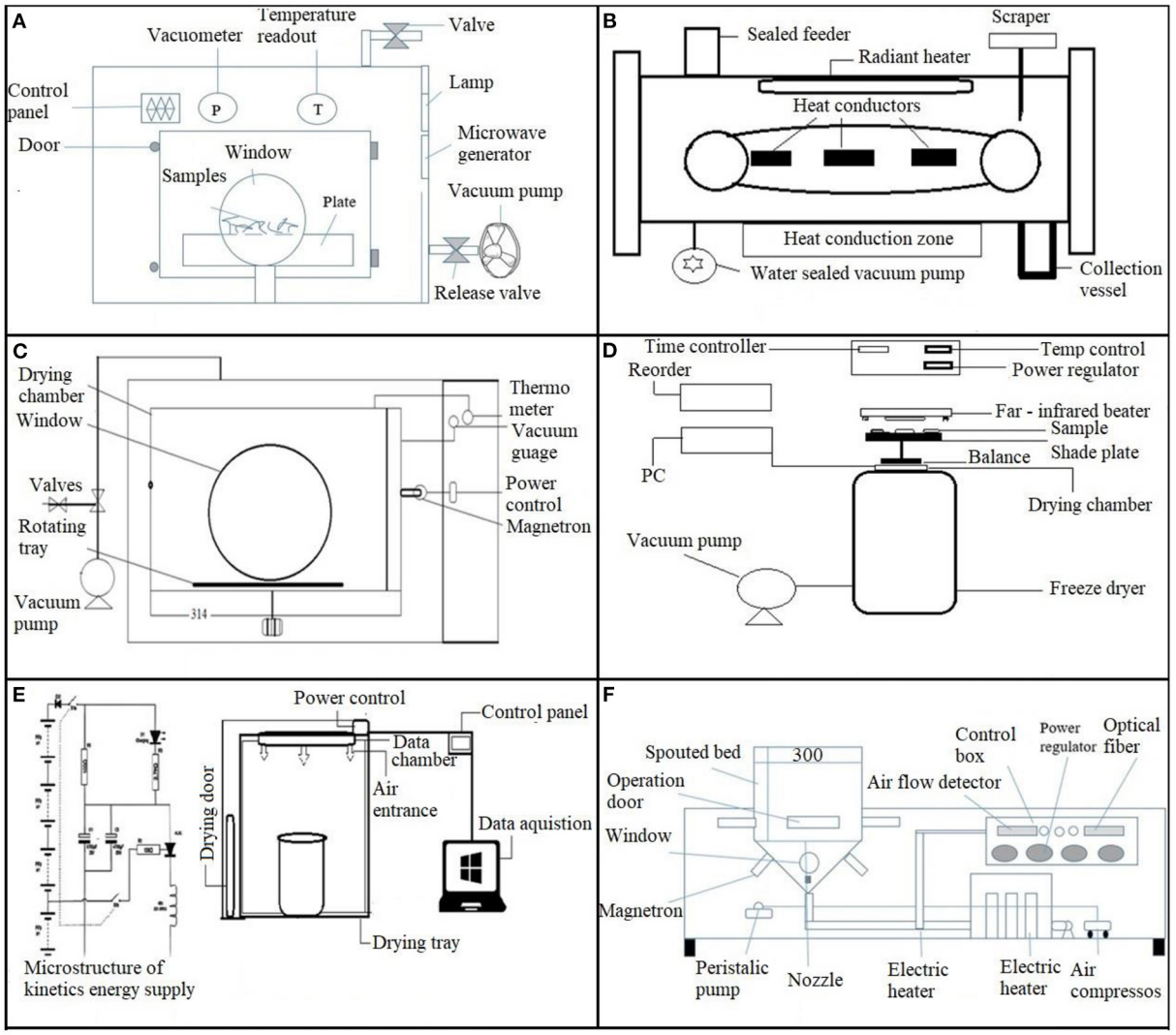

FIGURE 5
Special purpose drying techniques are used for sweet potatoes. **(A)** Vacuum microwave dryer. **(B)** Continuous vacuum belt dryer. **(C)** Microwave vacuum dryer. **(D)** Freeze dryer and far-infrared dryer. **(E)** Combined hot air and infrared dryer. **(F)** Microwave spouted bed dryer.

Where ES denotes specific energy consumption (kJ/kg), E denotes total energy consumption (kJ), and M represents the weight of moisture reduced from the samples (kg).

Microwave vacuum drying

It is a new technology that operates microwave radiation as a heat source in a sub-climatic pressure environment (Figure 5A) and exhibits the benefits of both microwave and vacuum drying. Likewise, sublimation drying, vacuum and microwave energy's instantaneous and direct volume heating, and low drying temperature can both increase energy efficiency and quality of products (76). Marzuki et al. (77) revealed that PFSP dried in 6–12 mins under microwave vacuum drying (MVD) conditions, significantly faster than hot-air drying (600 mins) and total phenolic content (TPC), color, and antioxidant activity improved. Similar findings were described by Lagnika et al. (78), who found that anthocyanins and total phenolics were abundant in PFSP. Correspondingly, total carotenoid and vitamin-C levels were abundant in OFSP. As microwave vacuum drying produces excellent nutritional and sensory quality food with slight shrinkage. Monteiro et al. (79) found that MVD is an appropriate procedure for making highly porous sweet potato chips, enhancing the value and prolonging the shelf life of vegetables. Furthermore, research on the bioactive components of purple and orange sweet potato slices impacted by MVD after pretreatment is restricted. This knowledge gap may affect the

efficient production of premium products from these two sweet potato varieties.

The following equation can calculate microwave-vacuum drying's energy efficiency:

$$DF = \frac{t_{on}p(1 - m_f)10^{-6}}{M_i(m_i - m_f)} \quad (11)$$

Where t_{on} (s) = microwave drying exposure time at the applied power input, P (W), Mi = the sample's initial mass (kg). The initial and final moisture levels are m*i* (kg) and m_f(kg), respectively.

Catalytic infrared drying

The catalytic infrared drying (CIR) emitter is motorized by propane or petroleum gas to generate thermal radiant energy *via* a synergistic reaction with a catalyst pad inside the CIR transmitter (Figures 4E,F) (Table 3). The CIR emitter uses less energy than traditional infrared emitters, which use electricity to convert natural gas straight into radiant radiation (80). Manu et al. (81) used a flameless gas infrared catalytic drier to dry mango-sweet potato leather with a moisture content of 15.4% at 45, 50, and 55°C. The cabinet, oven, and solar-dried jack fruit leather moisture levels were 18.85, 14.79, and 18.5%, respectively (82). They determined that a catalytic infrared drier was more feasible in drying mango-sweet potato leather than a cabinet, oven, or sun dryer. Similarly, Rashid et al. (83) noted a faster drying rate in a CIR drier at 20 and 40 kHz US frequencies at 70°C compared to HAD at 60, 70, and 80°C for SPs dried by Onwude et al. (37).

The energy efficiency calculated by Coskun et al. (84) of an infrared dryer can be calculated below.

$$E_T = R_{il} + E_{cb} \quad (12)$$

where E_{il} is the energy consumed by infrared lamps and E_{cb} is the energy used by the centrifugal blower.

$$E_{il} = k \times t \quad (13)$$

k represents lamp power, and t represents drying time.

$$E_{cb} = (\frac{V^3}{16600}) \times t \quad (14)$$

V represents the air velocity (m/s).

Combine hot-air and infrared drying

Traditional drying processes are widely utilized in agricultural commodities to preserve and process agricultural goods. In particular, hot air drying (HAD) is extensively employed for commercial and industrial food preparation. It consumes a lot of energy and may impact the final product quality due to a longer drying time (38, 64). As a result, novel and inventive drying procedures have attracted considerable attention. It has been revealed that combining infrared and hot air-drying techniques (Figure 5E; Table 2) can increase energy efficiency and dried agricultural product's quality. However, research on its applicability to drying commercial crops, such as SPs, is limited because it is a new drying process. The only study conducted by Onwude et al. (37) reported that combining IR and HAD techniques resulted in shorter drying times and lower energy consumption than HAD and IR dryers. Meanwhile, different combinations of IR + HAD substantially influenced the phytochemical characteristics of dried SPs, resulting in improvements in TFC, TPC, and DPPH. Overall, the combination of IR and HAD showed significant potential, providing more valuable knowledge of the sweet potato drying process than traditional dryers.

Energy efficiency is calculated by dividing the energy required for moisture evaporation from the drying product by the total energy SEC consumed during the drying process.

$$\eta_e = (\frac{E_{evap}}{SEC}) \times 100 \quad (15)$$

where η_e denotes energy efficiency, and E_{evap} indicates the amount of energy required to evaporate moisture (kJ).

Continuous vacuum belt dryer/vacuum freeze-drying

A continuous vacuum belt drying system (Table 2) was developed so that the product moves along the belt under a vacuum and is heated by conduction or radiation (Figure 5B). It has been used to commercialize instant tea, high-quality citrus crystals, and medicines. The only study by Xu et al. (42) examined the application of comparatively low-temperature vacuum belt drying to eliminate water without significantly changing the phytonutrients of vegetables while creating a crisp structure. Crispy sweet potato chips with nice color preserve a significant amount of β-carotene and are preferred by customers can be produced *via* vacuum-belt drying at 100–120°C or a specified temperature amalgamation.

The drying efficiency is the amount of heat energy used to evaporate moisture from fruits to the total energy consumed.

$$\eta = \frac{M_w.L}{E_T} \quad (16)$$

Where MW = mass of water evaporated in kg, and L = latent heat of vaporization in KJ/kg.

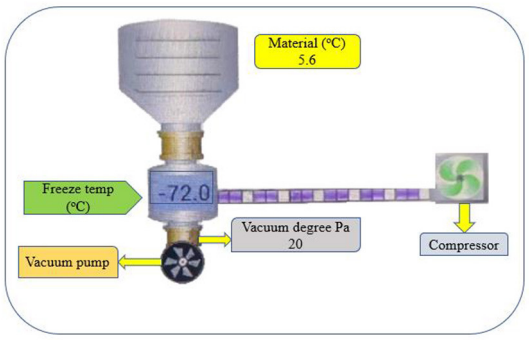

FIGURE 6
The mechanism of vacuum freeze drying.

Vacuum freeze-drying (VFD) is a technique for solidified materials drying by water sublimation under a vacuum (Figure 6; Table 2). It is the best method for removing water from the final product in comparison with other drying techniques (85). VFD highly preserved β-carotene in SPs compared to microwave and hot-air drying (HAD), but VFD and HAD require days to dry samples, whereas microwaves can dry in only minutes. Yang et al. (85) further noted that microwave and hot air drying provided more phenolics and antioxidants. Similarly, the drying time in VFD improved the brightness of purple SPs but had little effect on other color variables (86). Deng and Jiang (87) concluded that VFD has the lowest comprehensive effect on sweet potato flour than HAD and vacuum drying (Figure 5D). Therefore, VFD is the most cost-effective method of obtaining sweet potato flour and requires further exploration.

The energy efficiency of vacuum drying can be calculated as below,

$$E_T = E_h + E_r + E_c + E_v + E_s \quad (17)$$

E_h = denotes hydraulic energy.
E_r = stands for refrigeration energy.
E_c = stands for circulatory energy.
E_v = is the vacuum's energy.
E_s = stands for energy for sublimation.

Drying pretreatments

To reduce nutritional loss and enhance the quality of dried food materials, pretreatments are commonly used with the drying method. The effects of chemical and osmosis pretreatment on dry SPs' nutritional and bioactive properties are summarized below and in Table 3.

Chemical pretreatments

These can significantly improve drying kinetics; however, they can also result in the loss of soluble nutrients and chemical residues, producing food safety issues.

Citric acid is an organic acid used in fruits and vegetables as a texture modifier for anti-browning treatments. Meanwhile, citric acid has been shown to speed up drying because pectin loosens in an acidic environment, allowing water to be removed (88). Table 1 highlights the effect of CA pretreatment on sweet potato color retention and drying rate. Singh et al. (63) discovered that pieces of sweet potato treated with 1% CA at 50°C had improved color and required less energy to dry. Another study found that pieces treated with KMS (1.0%) (Potassium Metabisulfite) and CA (1%) at 50°C had improved color with less energy to dry (89). On the other hand, treatment with 1% CA preserved fewer bioactive compounds and antioxidants after drying at 55°C than 3% CA treatment, showing that 3% CA pretreatment resulted in better preservation effects (90).

Sulfidation, often known as sulfuring, is a standard technique for reducing obscuring throughout drying and maintaining quality during food processing and storage on an industrial scale (25). Commonly utilized sulfur dioxide gas or water-soluble sulfide salts include potassium metabisulfite ($K_2S_2O_5$), sodium metabisulfite ($Na_2S_2O_5$), and sodium hydrogen sulfite ($NaHSO_3$). The drying methods (hot-air and drum dryer) increased the anthocyanin content of SPs by 1.8 to 3.8 times by pretreatment with (0.5% w/v) sodium metabisulfite; however, the drying process resulted in a considerable loss of β-carotene. Compared to hot-air drying, drum drying produces sweet potato flour (SPF) with superior color, TPC, and antioxidant activity (91). Desulfurization treatment can improve the quality of SPs, such as rehydration ratio, β-carotene content, and color (35, 63). The quality improvements in sweet potato chips after potassium metabisulfite and sodium chloride treatment (92). The flour quality of OFSP was also increased by sodium hydrogen sulfite solution (50).

The carbonic maceration (CM) strategy is used in its general application. It involves placing samples in a carbon dioxide-rich sealed tank an adaptation that is immediately reflected in the transition of plant internal materials from respiratory to fermentative anaerobic digestion (93). Mainly, pretreatments of CM have been used in drying chili and raisins (94, 95), respectively. However, only one study explores the impact of CM pretreatment on sweet potato drying behavior. SPs were processed with CM, which upgraded the drying procedure and improved the dried good quality. CM pretreatment reduced sweet potato drying time by 38.1–34.6%, and the phytochemicals (phenols, anthocyanin, flavonoids, -carotene substance, and ascorbic acid) and DPPH radical activity was 13.83–78.18 and 10.04–14.09% higher than those of untreated samples, respectively (45).

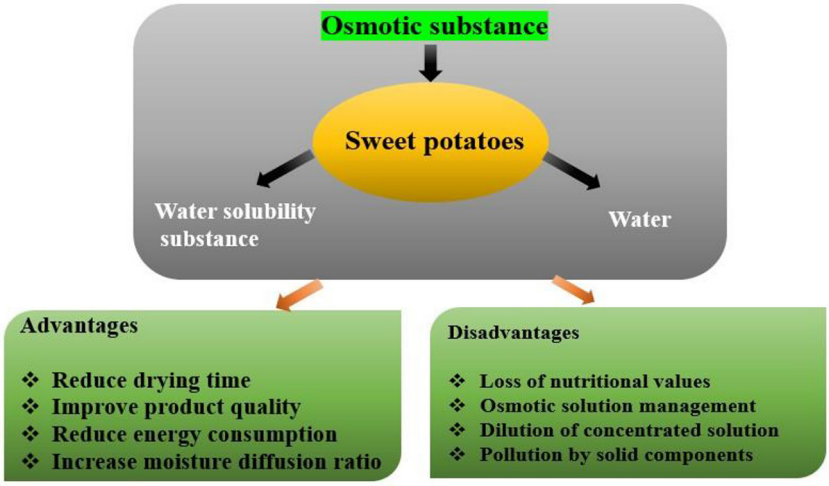

FIGURE 7
Osmotic dehydration principle of sweet potato drying.

NaCl is used as an antioxidant, improving sulfurous acid's antioxidative activity. NaCl also restrains the movement of oxidizing catalysts, for example, the polyphenol involved in peeling and cutting fruits and vegetables with discoloration (96). Osmotic solutions, such as salt, mainly reduce SPs' drying time and product quality. The same trend was noted by Singh et al. (92) for the quality enhancement of sweet potato chips using NaCl as an osmotic agent. On the other hand, the salt solution had no leaching effect on β-carotene and acted as an antioxidant agent for β-carotene in SPs (54). On other aspects of SPs in a NaCl solution and found that the salt solution reduced the drying time and effective diffusivity according to Fick's equation diverse from 3.82×10^{-10} to 7.46×10^{-10} m^2/s for water and 1.18×10^{-9} to 3.38×10^{-10} m^2/s for solids (55). Similar findings were confirmed by Clifford et al. (54), who reported that salt solution could be an energy-saving cost for the dehydration of SPs.

Osmotic pretreatments

Osmotic dehydration removes water from fresh food by immersing it in a solution with high permeation pressure, which has less water activity (Table 4). As demonstrated in Figure 7, during osmotic pretreatments, the cellular structure works as a semi-permeable barrier, allowing for countercurrent mass transfer. As the solute enters the products, internal moisture is transferred to the hypertonic solution (97). Researchers have compared different types of osmotic solutions, such as sucrose, glucose, fructose, and sorbitol (54, 56, 57, 89, 98–103).

Sucrose is the most often used osmotic agent in sugar pretreatments due to its low cost and high mass transfer (104). Adding sucrose to a product improves the dried product's sweetness and calorie content. Substances e.g., fructose and sorbitol may be utilized to minimize water activity. Fruit-derived agents have smaller molecular weights than sucrose, a sweet flavor, and lesser calories (105–107). They inhibit enzymatic and non-enzymatic browning activities, reducing energy consumption while preserving color and smell. They also remove surface and intercellular gases, which prevent oxidation, browning, softness, and off-flavor formation. Most sweet potato OD studies used sucrose and NaCl as osmotic agents (55). There have been few studies on the nutritional aspects, shrinkage, and drying kinetics that occur throughout the process and non-ionic agents like sorbitol, glucose, and fructose for the OD of sweet potato. One of the studies by Brochier et al. (106) observed that OD (sucrose, sorbitol, fructose) was an excellent pretreatment for microwave drying of SPs with a shorter drying time. Weibull models gave a best-fit model for microwave drying.

Similarly, de Junqueira et al. (89) found sorbitol as the finest osmotic agent, with the most significant water loss and the minimum gain of solid. Fructose was more efficient at reducing water activity ($a_{w)}$ in the samples, although it resulted in more solid absorption. The absolute sugar concentrations of the SPs studied ranged from 4.8 to 12.5%, with glucose, fructose, and sucrose levels in fresh roots varying between genotypes (108).

Ultrasound pretreatments

Ultrasound (US) is a developing technology in the food industry because it has many advantages over traditional food processing methods (Table 4). The sponge effect was also induced by ultrasound, which created a fast alternate

compression and expansion of the food matrix. Sonication also generates cavitation in a liquid medium, forming bubbles that can explode and cause confined pressure and temperature expansion (Figure 8). Compared to untreated samples, US pretreatment might enhance the β-carotene concentration by 4–42%. As a result, pretreatments developed in the US can increase the quality of SPs though lowering the drying period (109). Tayyab Rashid et al. (1) conducted a series of studies on the ultrasonic drying of SPs. They found that vitamin C was better maintained in the combination ultrasound/glucose treatment, while antioxidant assays were more significant in ultrasound- and glucose-pretreated samples. The effective moisture diffusivity was raised by conducting ultrasound pretreatment to minimize drying duration.

In contrast, according to the findings of ultrasonic-osmotic dehydration, the osmotic solution did not influence moisture diffusivity. Among the various US pretreatment timings, the 30 min ultrasonically osmotic treatment (US/GC-10%-3) was the most effective drying time reduction (100). In another study of multi-frequency ultrasound with infrared drying, an optimized US frequency (40 kHz) at 70°C reduced the drying time to 20 kHz, resulting in higher phytochemical quality in dried samples than in fresh samples (101). Similarly, pretreatment with ultrasound at 40 kHz (70°C) retained the phytochemicals in dried SPs. According to HPLC analysis, the most common phenolic acids were ellagic and chlorogenic (44). The ultrasound treatment had a great impact on the morphology of sweet potatoes for its cavitation and turbulence effect. Lv et al. (110) also reported that generated debris and irregular pores of the ultrasound-treated egg white sample were due to ultrasonic cavitation and the mechanical effect. This led to an increase in water loss and solid gain and the more the water loss and solid gain, the more collapse the cell structure (111). This finding is similar to those reported on egg white by using ultrasound (112). The ultrasound-treated sweet potato samples considerably reduced drying duration from 110 to 60 min in contrast with the control samples. Amongst the 13 explored mathematical models, the Hii, Page, and Silva models adequately reflected drying kinetics (1).

The effect of drying techniques on sweet potato nutritional qualities

Drying can dramatically change food products' phytochemical properties and other quality attributes. The main characteristics of SPs that must be considered when drying are their proximate composition (fat, fiber, carbohydrate, ash, and protein), vitamins, minerals, anti-nutrients, sensory characteristics, and antioxidant properties (phenols, flavonols, ABTS, DPPH). In general, drying increases the nutritional value (83).

Drying methods, time, and temperature significantly affect SPs' nutritional value. Fat, vitamin C, protein, total carotenoids, and beta-carotene decrease with drying temperatures, while mineral, fiber, ash, and carbohydrate content increase significantly (50, 113). Studies on the proximate composition of SPs revealed that fresh samples had substantially lower proximate composition (protein, fat, fiber, and carbohydrate) and mineral content compared to dried samples because of the significant amount of moisture loss during drying, leading to a rise in the level of other nutrients (113–115). Sun-drying (40, 114, 116) and oven drying (50, 115) are the best methods for preserving the quality of dried SPs. Because of their short drying times, infrared and microwave drying was the most cost-effective of all drying technologies. Onwude et al. (37) revealed that average SEC values obtained were lesser than those acquired for other fruits dried by various drying techniques e.g., convective hot-air drying of mushrooms (47.88–93.45 kW h/kg) (117), vacuum drying of mushrooms (41.97–124.34 kW h/kg) (117), and combined microwave and hot-air drying of longan (8.23–10.08 kW h/kg). Under quicker drying conditions, the qualitative characteristics of SPs used for food were better retained.

Minerals and vitamins are essential nutrients required by our body to function correctly. As with the approximate ingredients, minerals and vitamins significantly affect the drying time, drying method, and drying temperature. Kosambo (70) stated that cabinet drying usually retains more ascorbic acid than sun drying. The loss of trans-carotene (Provitamin A) during sun-drying was higher than that of cabinet drying in SPs. There is little difference between the preservation of vitamin A in the tunnel and the open-air solar dryer of SPs (13 and 10%, respectively) (16). According to Bechoff (35), hot-air crossflow drying preserves more vitamin A than sun-drying. In addition to magnesium, the temperature positively impacts the mineral quality of SPs. The discrete mineral composition of SP flour has seldom been deliberated, whereas the amount of ash is usually reported as the estimated total mineral content. Results from Olatunde et al. (118) indicate that sweet potato roots are a good source of minerals, particularly essential micronutrients like Cu, Zn, iron, and Mn.

Mathematical models used in drying sweet potatoes

The drying process modeling is a critical component of drying technology, particularly in industrial operations. The essential characteristics of thin layer drying technology are the mathematical modeling of the drying procedure and the design of equipment to select the most suitable operating conditions. These models are often used to describe dried SPs. The developed model has been used for calculations, including designing, and constructing new drying systems, optimizing the

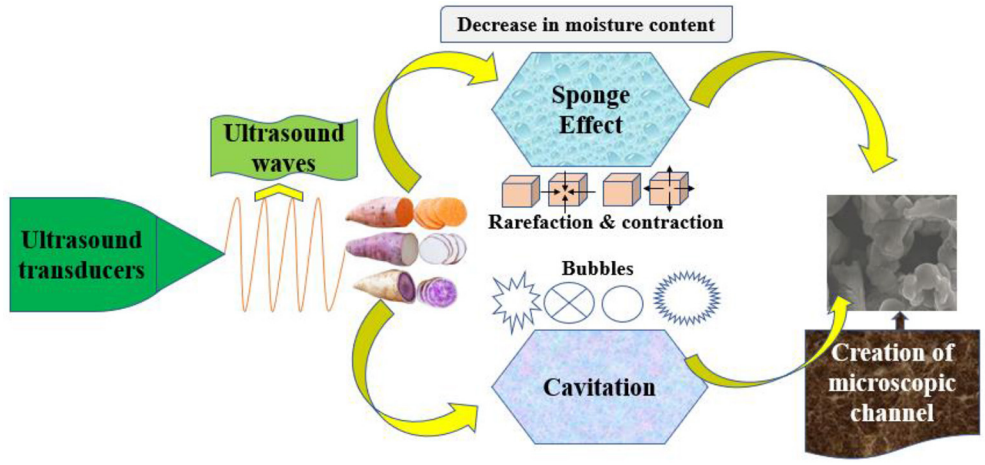

FIGURE 8
Ultrasound impact on sweet potatoes samples.

drying techniques, and describing the drying behavior, including combined macroscopic-microscopic media for mass and heat transmission. Drying conditions, dryer type, and the material's properties to be dried all affect drying kinetics. These actions speed up the drying process since the material is completely subjected to hot air and temperature drying conditions. These models account for exterior resistance to the moisture transport method between the atmosphere and material, providing a greater degree of accuracy, better predicting the behavior of the drying process, and making fewer assumptions as they rely on experimental information. Therefore, these models have proven most valuable to dryer engineers and designers (11). Though, they are only efficient under particular dry circumstances. Alternatively, the theoretical models include numerous hypotheses, leading to many errors and restricting their use in dryer design (18). To fit the drying data, various drying models were applied. The model parameters k, n, and a were determined by fitting the curve. The determination coefficient was utilized to evaluate the experimental data's fit (R^2). The model with the least possible RMSE and chi-square χ^2 and the uppermost R^2 was chosen as the best fit for sweet potato thin layer drying characteristics. The better the fit, the greater the R^2 value. Generally, an R^2 value of 0.97 or above is considered an excellent match (36, 37). Table 5 summarizes the mathematical models suitable for drying various SPs.

Activation energy (E_a)

E_a is the least amount of energy needed for drying. It is assessed from the association between sample average temperature and effective moisture diffusivity by following Arrhenius Equation (18):

$$D = D_0 \exp\left(-\frac{E_a}{R(T + 273.15)}\right) \quad (18)$$

D_0 denotes the diffusion factor (m^2/s), R = universal gas constant (8.3145 kJ/mol). E_a = activation energy (kJ/mol), and T = sample's average temperature (135, 136). Equation (19) predicted the E_a values for various product thicknesses by drawing the fitting curve between ln D and 1/(T + 273.15).

$$Slope = -\frac{E_a}{R} \quad (19)$$

E_a values of the SPs are tabulated in Table 6. The *Ea* results summarized in Table 6 are within the acceptable range of 12–43.26 kJ/mol for vegetables and fruits (6). Greater activation energy derives from the increased energy needed to commence moisture diffusion in a large-thick material.

Specific energy consumption

When selecting a proper drying technique to minimize process costs, energy consumption during various drying methods should be considered. According to Xie et al. (139), the lowest and greater specific energy consumption were 81.537 and 173.761 (MJ kg^{-1} water) at infrared drying at 70°C, respectively. The lowest specific energy consumption (SEC) value (0.680 MJ kg^{-1} water) for drying samples was attained with a thickness of 3.5 m at a microwave power level of 200 W. Whereas, the highest value (2.591 MJ kg^{-1} water) was achieved 9 mm thick sample and a power level of 800 W (138).

TABLE 5 Thin layer models were reported in previous studies for sweet potato drying.

S. no.	Model name	Model	References
1	Newton	$Mr = \exp(-kt)$	(119)
2	Page	$Mr = \exp(-kt^n)$	(120)
3	Modified page 1	$Mr = a\exp(-kt^n)$	(23)
4	Handerson and Pabis	$Mr = aexp(-kt^n)$	(121)
5	Modified Henderson and Pabis	$Mr = aexp\left(-kt\right) + bexp\left(-gt\right) + cexp(-ht)$	(122)
6	Logarithmic	$Mr = aexp\left(-kt\right) + c$	(123)
7	Midilli	$Mr = aexp\left(-kt\right) + bt$	(124)
8	Two-term	$Mr = aexp\left(-k_1t\right) + bexp\left(-k_2t\right)$	(125)
9	Two-term exponential	$Mr = aexp\left(-kt\right) + (1-a)\exp\left(-kat\right)$	(36)
10	Hii	$Mr = aexp\left(-k_1t^n\right) + bexp\left(-k_2t^n\right)$	(126)
11	Verma	$Mr = aexp\left(-kt\right) + (1-a)\exp(-gt)$	(127)
12	Modified Midilli	$Mr = aexp\left(-kt\right) + b$	(128)
13	Aghbashlo	$Mr = \exp\left(k_1t/1 + k_2t\right)$	(129)
14	Wang and Singh	$Mr = 1 + at + bt^2$	(130)
15	Silva	$Mr = \exp(-at - b\sqrt{t})$	(131)
16	Jena and Das	$Mr = aexp\left(-kt + bt^{\frac{1}{2}}\right) + C$	(132)
17	Parabolic	$Mr = a + bt + ct^2$	(133)
18	Weibull model	$Mr = a - b\ \exp(-kt^n)$	(134)
19	Approximation of diffusion	$Mr = aexp\left(-kt\right) + (1-a)\exp(-kbt)$	(36)

When drying SP, Onwude et al. (8) convective hot-air drying can consume ∼337.79 (MJ kg^{-1} water) of specific energy at 70°C for 4 mm slices. According to other research findings, a lower drying temperature results in higher specific energy consumption (142). SEC was 227.39 and 265.99 (MJ kg^{-1} water) at 50 and 60°C, respectively. Infrared-assisted convective hot-air drying, on the other hand, reduced energy consumption by 69.34–85.59% (8). Limited investigations have assessed the specific energy consumption of enhanced drying approaches, such as microwave-assisted convective drying, particularly when drying SPs. Further study should be conducted using combined drying methods to determine SPs' specific energy consumption. However, SER is stated as the amount of energy consumption (EC) on the dry basis to sample mass MS (g), or the EC ratio removed from the sample during drying to water mass MW (g)

$$SER = \frac{E_C}{m_s} \tag{20}$$

$$SER = \frac{E_C}{m_w} \tag{21}$$

During the drying process, the SER value slowly declines as the moisture removal is significantly reduced as a result of inadequate moisture of the dried product surface.

Major issues and future recommendation

The positive aspects of a controlled drying method include rapid drying, preservation of nutrients, color, and shelf life.

A few future research hotspots are found in detailed literature surveys mentioned below.

1. Optimization conditions could be used in dryers and quality parameters to diminish the drying time and make higher-quality foodstuffs more attractive to product developers and consumers.
2. Osmotic pretreatments, such as sugars, improve the nutritional quality while making the product difficult to dry.
3. Using US application alone or combined with osmotic pretreatment enhances the mass transfer ratio with minimal reduction in the structure and quality of SPs.
4. Combined dryers including hot air, IR, and microwave drying reduce the drying time and cost of regular dryers, i.e., cabinet or oven drying.
5. Further studies on improving quality parameters and mathematical modeling, hyperspectral imaging, FTIR, and NIR techniques might be employed as an alternative non-destructive tool for fast, accurate, and rapid determination in the drying process.

TABLE 6 Activation energy and energy consumption summarized from previous studies for sweet potato drying.

Drying conditions/Temperature (°C)	Size (mm)	R^2	Activation energy E_a (KJ/mol)	Energy consumption (MJ.kg^{-1} water)	References
CHAD, IRD, IRD-CHAD; T = 50–70°C, 1,100–1,400 W/m^2 IR	4–6	0.784–0.999	13.24–14.87, 12.22–18.76, 11.57–36.44	6 mm CHAD 220.39, IRD 0.34 kW h/kg 4 mm CHAD 337.79, 2.06 IRD	(34)
IR = (1,100 and 1,400 W/m^2)	4–6	0.999	12.83–34.64	Varied from 0.91 to 4.82	(34)
HAD; T = 50–80°C	5, 10, 15	0.987	11.1, 30.4	–	(18)
HAD; T = 40–70°C; US = 0, 30, 60 W	5	–	–	Varied from 1.4 to 2.6	(47)
HAD; T = 50–80°C SMB, WAT, UNT treated	4	–	11.25, 9.13, 17.5	–	(49)
100 Pa MWFD, 4.5 kPa MWVD, 80°C MWSBD	10	–	–	MWFD 10,027.33, MWVD 4,259.33, MWSBD 3,004.33	(137)
HAD; T = 10–40°C	–	–	64.2	–	(35)
P = 200, 400, 600, 800 W	3.5, 5, 7, 9	–	1.621, 1.597, 1.451 and 1.423	Minimum 0.680 and maximum 2.591	(138)
HAD; T = 50–90°C	5, 8, 12	0.990	6.18–19.04	–	(14)
IR; T = 70°C	3	–	–	Minimum 81.537 and maximum 173.761	(139)
HAD; T= 50–90°C	3–8	–	13.48–16.50		(13)
Far-IR; T = 60, 70, 80°C	8, 10	–	–	8 mm was <2.42–3.41	(140)
HAD; T = 50–80°C	–	0.987	23.29	–	(17)
Tray, IR, FBD; T = 45, 55, 65°C	–	–	35.88, 33.21, and 17.33	–	(36)
HAD; T = 50–70°C	-	–	23.2 and 22.7	–	(64)
IR-HAD; T = 60–70°C	4	–	11.38	27.67–41.44	(37)
HAD; T = 50, 60, 70°C; IR = 1,100 W/m^2	4	–	8.74–34.76	–	(141)
MWFD, WVD, MWSBD (−38, 4.5 KPa, 80°C)	10	–	–	–	(137)

6. Further research is needed to improve model parameters or modify existing models, such as heat transfer coefficients and standardized experimental temperature measurement methods, and apply the development model to SPs.
7. Due to the increase in experimental costs, the focus is on computer-based solutions, namely online approximation of drying kinetics and drying techniques that also need to control industrial operations. To achieve more dependable and precise findings, the correctness of these simulations was determined by utilizing a suitable mathematical model for drying.

Conclusion

This review examines sweet potato drying methods and offers the findings of previous studies. The various drying processes, drying rates, and impacts on product quality are outlined, and operational requirements for increasing drying quality are mentioned. This detailed study includes mathematical models for estimating sweet potato water ratios. A greater drying temperature, lower relative humidity, and better velocity contribute to a faster drying rate. The drying speed of sweet potatoes is also affected by their quality qualities. Osmosis dehydration minimizes the drying time, initial water content, energy consumption, and product quality and. chemical pretreatments can improve the drying procedure and uphold food quality. Furthermore, novel non-thermal methods, such as ultrasonic waves, have been developed as alternative pretreatments for reducing drying periods and improving quality.

Natural drying procedures, such as sun, shadow, and wind drying, are low-cost and ecologically benign. Electric heating dryers (oven dryers) consume a lot of energy and only have

a limited impact on product quality. Infrared and microwave dryers are resources and provide total control over the quality of dried items. The relative humidity and drying temperatures can be modified to get the final product with minimal nutritional loss. Low relative humidity, medium air temperatures (40–70°C), and high velocity are critical operating parameters for an effective drying operation. Several scholars have presented mathematical models of sweet potato drying, including the Page, Hii, and Midilli models, which give the optimum fitting when characterizing sweet potato drying behavior. Most recent research has found that drying sweet potatoes at lower temperatures preserves their nutrients.

The future challenge in food drying is to optimize the dryers and reduce both the equipment cost and running cost to improve the quality of the product and to use renewable energy for drying to reduce emissions. As the expense of trials rises, more emphasis is being placed on computer-based solutions such as FTIR online assessment of quality parameters, NIR spectroscopy to simulate drying difficulties, and selecting the appropriate dryer.

Author contributions

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

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Antioxidant and antidiabetic activities of a polyphenol rich extract obtained from *Abelmoschus esculentus* (okra) seeds using optimized conditions in microwave-assisted extraction (MAE)

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Functional foods have gained popularity in recent decades. They are exploited for their bioactive compounds like polyphenols, which are highly demanded in cosmetic, pharmaceutical and nutraceutical industries. However, extractive techniques and conditions used up to recently are almost obsolete and must be optimized for higher efficiency. The current study aimed to evaluate the antidiabetic potential of an optimized extract of *Abelmoschus esculentus* (okra) seeds. The optimal conditions for extracting polyphenolic compounds from okra seeds were determined using Microwave Assisted Extraction (MAE). A Face Center Composite Design (FCCD) was used for optimization. Solvent/dry matter ratio, wavelength and time were considered while the response studied was the polyphenolic content. The extract obtained at optimal conditions was characterized using Thin Layer Chromatography (TLC) and Fourier Transform Infra-Red (FTIR) spectroscopy, then tested for its antioxidant, alpha amylase inhibitory and antidiabetic activities. Response Surface Methodology (RSM) permitted the determination of the optimal conditions for phenols extraction as: microwave power 330 W, with a solvent ratio of 97.04/1 mL/g for 9.5 min of extraction time. The optimized extract showed a phenolic content up to 86.37 ± 1.13 mg GAE/g containing quercetin and catechin as revealed by the TLC. Functional groups characteristic of polyphenols were identified on FTIR spectra, and the extract exhibited good *in vitro* antioxidant capacities with DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging capacity and FRAP (Ferric Reducing Antioxidant Power Assay). An IC_{50} of 3.99 ± 0.15 μg/mL was obtained with the DPPH scavenging test. Alpha amylase inhibitory assay revealed that the optimized okra extract behaved as a non-competitive inhibitor of porcine pancreatic amylase with an IC_{50} of 484.17 ± 2.33 μg/mL.

Antidiabetic activity of the extract was observed in streptozotocin-induced diabetic males Wistar rats, as shown by the fasting blood glucose levels, food intake, changes in body weight and serum lipid profile among others.

KEYWORDS

optimization, phenol, diabetes mellitus, okra, microwave assisted extraction

Introduction

Considerable adverse side effects of oral antidiabetics used up to date have reinforced the interest of scientists and patients in functional foods and their derivatives for the management of many chronic diseases (1). *Abelmoschus esculentus* (okra) fruits, for example, have long been investigated for their antidiabetic potential. Seeds, peels or the whole fruits have shown their efficacy in reducing blood glucose levels in experimental animals. Authors mostly attributed the antidiabetic activity of okra fruits and other plants to their polyphenolic content (2). However, the extraction techniques that had been used seem rudimentary compared to what is done nowadays (3). Content of bioactive compounds obtained by traditional extraction methods such as distillation, maceration and solvent extraction among others, used by the previous researchers can be highly improved by cutting-edge techniques like pressurized hot water (PHWE), microwave-assisted (MAE) and ultrasound-assisted (UAE) extractions, thus allowing a potentially great amelioration in the antidiabetic activity of okra fruits or parts. Also the extraction time is significantly reduced by these new techniques (4, 5). Meanwhile each extraction technique has its own advantages and limitations; MAE has demonstrated its high efficiency for a fast extraction of good quality bioactive compounds from natural sources when the solvent is well chosen (6). Okra seeds have been reported as the richest part of the fruits in polyphenols and flavonoids in general, and also in demonstrated antidiabetic compounds such as quercetin and its derivatives in particular (3).

Flavonoids, just like other phenolic compounds are also known to have antioxidants, anti-inflammatory, anti-cancer activities among others (7) and are highly demanded nowadays by cosmetic, pharmaceutical and nutraceutical industries. So, determination of experimental conditions for maximum extraction of polyphenolic compounds from okra seeds using a more efficient technique is quite urgent in order to reduce pollution, solvent and time wastage and above all improve the extraction yield, the quality and the bioactivity of the extracts (8–10). Geng et al. (11) have determined the optimal conditions for extracting polyphenols from okra flowers using conventional maceration technique while Amirabbasi et al. (6) established the conditions for a maximal extraction of polyphenols from okra stems using MAE and UAE. This study aimed to determine the optimal conditions for extraction of polyphenols from okra seeds by a face centered composite design, using MAE and to evaluate the antidiabetic activity of the extract obtained.

Materials and methods

Material

Plant material

Mature fruits of *Abelmoschus esculentus* were harvested from our botanical garden situated in Dschang (5°27/ nord, 10° 04/ east) West region of Cameroon. The fruits were shade dried and the pods opened for seed collection.

Chemicals

All reagents were purchased from local stores while streptozotocin was provided by Sigma-Aldrich (Hamburg; Germany).

Experimental animals

To evaluate the antidiabetic activity of the optimized extract, twenty four 24 Wistar rats were collected from the animal house of the department of Biochemistry (University of Dschang) and placed in the animal room of the research unit. For 1 week, rats were fed a normal diet and water for acclimatization. All the animals (after dividing into groups) were housed in individual cages in the animal room under optimal conditions of light (12/12 light and dark cycle), temperature (27 ± 2°C), relative humidity (60 ± 10%) and a pathogen-free surrounding.

Method

Preparation of sample

Seeds of *Abelmoschus esculentus* were dried in an oven at 45°C until constant weight (24 h), and then finely ground using an electrical grinder (royalty line, 800 W, five cycles of 1 min each at full power). Powders were sieved (using a 500 μm sieve) and immediately used for extraction of phenolic compounds.

Choosing factors affecting the phenolic content

Based on the literature, the following factors were considered: time of extraction, dry matter/solvent ratio and the power of the microwave oven. A 50:50 hydroethanolic solution was used as solvent system, based on previous works (12, 13). Also, literature indicates that the solvent's dielectric properties should be highly considered when planning to extract phenolic compounds using MAE. Compared to water, ethanol or its mixtures with water have a lower dielectric constant, and are more transparent to microwave, thus not well converting microwaves into heat, but have high capacity to dissolve and extract phenolic compounds (14). Preliminary studies permitted to determine the ranges used for each factors.

Determination of responses

For each trial, 1 g of seeds powder was mixed with the appropriate amount of solvent according to the experimental conditions as given by the chosen design. The mixture was stirred using a magnetic agitator, afterward, it was allowed to rest for 10 min at room temperature and put in a microwave oven (SAMSUNG M735) for extraction, under specified conditions. Samples were centrifuged (4000 rpm /5 min) and the supernatant was collected after filtration through Whatman paper n°1. Solvent was then evaporated in an air oven at 45°C until obtention of the dry extract. Dry extracts were immediately used for determination of polyphenolic content. Figure 1 depicts the global flowchart of the work.

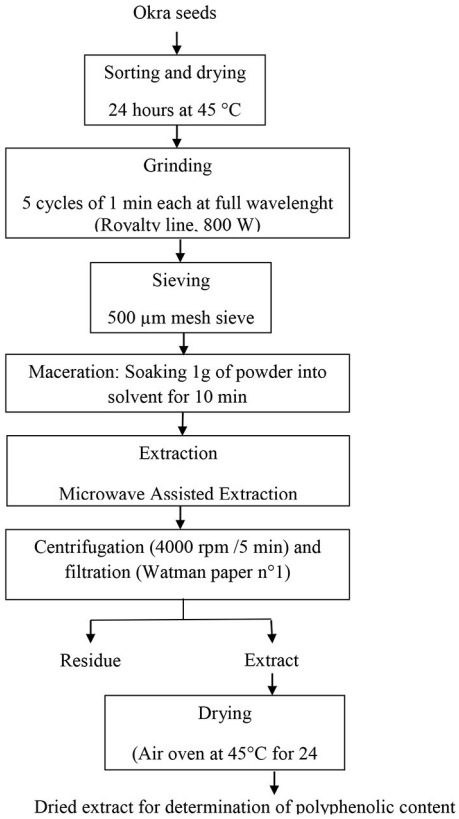


FIGURE 1
Extraction flowchart of phenols from okra seeds.

Determination of total phenolic content

The total phenolic content was assessed according to the protocol described by (15). Briefly, 0.2 mL of Folin reagent (ten-fold diluted) was added to a tube containing 0.01 mL of plant extract (5 mg/mL) and 1.39 mL of distilled water. The mixture was allowed to stand for 3 min before addition of 0.4 mL of sodium carbonate (20% w/v), and then mixed using a vortex. The tube was then incubated at 40°C for 20 min in a water bath and absorbance was read at 760 nm against a blank using a BIOMATE spectrophotometer. Gallic acid (0.2 g/l) was used to draw a calibration curve. All experiments were carried out in triplicates and results were expressed as mg of gallic acid equivalent (GAE) per g of dry extract (mg GAE/g dry weight).

Optimization of the responses using the central composite design

A face Centered composite design was used to optimize the response, total phenolic content (Y_1). Ranges of different factors were taken based on literature (6). Experiments were randomized and responses evaluated in triplicates. The proposed model was:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}{X_1}^2 + a_{22}{X_2}^2 + a_{33}{X_3}^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3$$

Where Y is the response (phenolic content), X_1, X_2, X_3 are the studied factors, a_0 is the offset term while a_1, a_2, a_3 are linear effects, a_{11}, a_{22}, a_{33} the quadratic effects and a_{12}, a_{13}, a_{14}, a_{23}, a_{34} are interaction effects. Table 1 shows the experimental design in coded and real variables.

Characterization of the extract

Determination of the total flavonoid content

The Total Flavonoid Content (TFC) was obtained using the method described by (16). Sodium nitrite of 0.03 mL (5%) was added to a tube containing 1.49 mL of water and 0.1 mL of extract solution (5 g/mL). After 5 min, a volume (0.003 mL) of aluminum chloride (10%) was added to the tube and the mixture was allowed to rest for 6 min. Afterward, 0.3 mL of NaOH (1M), and 0.24 mL of distilled water were introduced into

TABLE 1 Experimental design in coded and real variables.

Trial	Matrix of real and coded variables		
	Time (min)	Solvent (mL/g)	Power (W)
1	4.00 (−1)	30.00 (−1)	180.00 (−1)
2	15.00 (+1)	30.00 (−1)	180.00 (−1)
3	4.00 (−1)	80.00 (+1)	180.00 (−1)
4	15.00 (+1)	80.00 (+1)	180.00 (−1)
5	4.00 (−1)	30.00 (−1)	480.00 (+1)
6	15.00 (+1)	30.00 (−1)	480.00 (+1)
7	4.00 (−1)	80.00 (+1)	480.00 (+1)
8	15.00 (+1)	80.00 (+1)	480.00 (+1)
9	0.25 (−1.68)	55.00(0)	330.00(0)
10	18.74 (+1.68)	55.00(0)	330.00(0)
11	9.50(0)	12.95 (−1.68)	330.00(0)
12	9.50(0)	97.04 (+1.68)	330.00(0)
13	9.50(0)	55.00(0)	77.73 (−1.68)
14	9.50(0)	55.00(0)	582.26 (+1.68)
15	**9.50(0)**	**55.00(0)**	**330.00(0)**
16	**9.50(0)**	**55.00(0)**	**330.00(0)**
17	**9.50(0)**	**55.00(0)**	**330.00(0)**
18	**9.50(0)**	**55.00(0)**	**330.00(0)**
19	**9.50(0)**	**55.00(0)**	**330.00(0)**
20	**9.50(0)**	**55.00(0)**	**330.00(0)**

Bold values are replicates of the center points.

the tube and mixed with a vortex before absorbance was read at 510 nm against a blank. The calibration curved was made using catechin. All experiments were made in triplicates, and results were expressed as mg of catechin equivalent per g of dry extract (mg CE/g of dry weight).

Determination of crude fiber content

Crude fibers were quantified using the *Ceramic Fiber Filter* method as described by (17). These extracts and powders were previously treated to remove lipids using hexane (24 h soaking of 6 g of extracts and powders in 30 mL of hexane with gentle stirring). Briefly, 100 mL of 1.25% H_2SO_4 was added to 1 g of lipid-free powder in a round bottom flask and the mixture boiled under reflux for 30 min. The hot solution was quickly filtered under suction and the insoluble matter washed several times with hot distilled water until it was acid free. It was quantitatively transferred into the flask and 100 mL of hot 1.25% sodium hydroxide (NaOH) solution was added and the mixture boiled again under reflux for 30 min before it was quickly filtered under suction. The soluble residue was washed with boiling water until it was base free. Afterwards, it was dried to constant weight in the oven at 105°C, cooled in a desiccator and weighed. The weighed sample (C1) was incinerated in a muffle furnace at 300°C for about 2 h, cooled in the desiccator and weight measurement repeated (C2). The loss in weight of sample on incineration was given by C1–C2 while the crude fiber content was expressed as follows:

$$\%\ \text{Crude fiber} = \frac{C1 - C2}{Weight\ of\ original\ sample} \times 100$$

Saponin content

The saponin content was estimated as previously described by Koziol (18). Briefly, 0.5 g of the formulation was introduced in a graduated test tube, and 5 mL of distilled water was added.

The tube was closed and vigourously shaken for 30s, and the foam height was immediately measured. The saponin content is linked to the foam height by the following formula:

$$Saponin(mg) = \frac{[(0{,}432)(\text{Foam height after 5 -10 s}) + 0{,}008]}{\text{Sample weight (g)}}$$

Zinc content

Zinc content was determined using the protocol established by Pauwels et al. (19). The sample (1 g) was carbonized at 450 °C for 2 h in an oven (Carbolite Eurotherm), then digested with 10 mL of nitric acid 1N during 30 min. after cooling down, the solution was filtered using Whatman N°1 filter paper in a 50 mL flat bottom flask. Distilled water is added to the filtrate to bring the final volume up to 50 mL. Filtrate (20 mL) + NH_4Cl (20 mL) + concentrated HCl (1 mL) + sodium sulfite (1 drop) + potassium ferrocyanate (1 mL; 0.5%) were mixed and the mixture allowed to rest for 5 min in dark before absorbance were read using UV-visible spectrophotometer at 650 nm.

Identification of chemical functions and the number of bioactive compounds

Fourier Transformed Infra-Red Spectroscopy (FTIR) was used to identify the main chemical groups present in the extract while TLC was used to estimates the number of bioactive compounds or groups of compounds present in the extract to identify some.

Fourier Transformed Infra-Red Spectroscopy

Spectra were collected at 4000–400 cm^{-1} using a FT-IR Spectrometer (Alpha, Bruker, Germany) on a diamond plate at 4 cm^{-1}. Two replicates spectra of 50 scans each were recorded. Raw spectra were corrected.

Thin layer chromatography (TLC)

TLC was performed using a pre-coated plate with 60F250 silica gel (MERCK). Two standards were used: catechin and

quercetin (1 mg dissolved in 50 ml of ethanol, centrifuged, and the supernatant used). Development was done for 20 min in a pre-saturated (30 min) rectangular development chamber. The mobile phase was made of ethyl acetate/formic acid/ glacial acetic acid/water. The plate was dried at 45 °C in air oven and visualized under UV light (254 nm). Bands were circled and Rf calculated.

Antioxidant activities

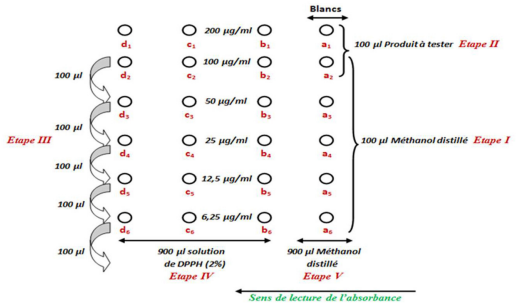
Ferric reducing ability of plasma (FRAP)

Ability of extracts and formulation to reduce ferric iron was tested as described by Oyaizu (20).

Briefly, 75 µL of extract/powder suspension was added to 2 mL of FRAP reagent (300 mM acetate buffer: pH 3.6; 10 mM TPTZ [(2, 4, 6-tris (2-pyridyl)-S-triazine)] in 400 mM of HCl; 10 mM ferric chloride). After 30 min of incubation at room temperature, the absorbance was read at 593 nm against a blank. BHT was used as standard.

DPPH inhibitory activity

DPPH inhibitory activity of the extract was assessed using a previously described method (21). Briefly, 100 mL of ethanol was introduced in tubes a_2-d_2, a_3-d_3, a_4-d_4, a_5-d_5, and a_6-d_6. Afterward, the extract was added in tubes a_1-d_1 and a_2-d_2. Dilutions were made starting from tubes a_2-d_2 to have final concentrations of 200 ; 100 ; 50 ; 25 ; 12,5 et 6,25 µg/mL. Finally, 900 µL of DPPH solution was added in tubes b_1-b_6, c_1-c_6 et d_1-d_6 while the same volume of ethanol was added in tubes a_1-a_6. The mixtures were allowed to stand for 30 min in dark before absorbance were read at 517 nm against blanks prepared in the same conditions (a_1-d_1).



DPPH inhibitory activities (%) were calculated using the formula:

$$\% = \frac{DOc - DOt}{DOc} \times 100$$

Where Doc = absorbance of the control; Dot = absorbance of the test.

Values of IC_{50} (concentration of product inhibiting 50% of DPPH°) were determined using percentages of antioxidant activities and were expressed in µg/mL.

Alpha amylase inhibitory activity and mechanism of action

The alpha amylase inhibitory activity was determined as described by (22) with slight modifications. Decimal dilutions of the extract were made ranging from 12.5 µg/mL to 200 µg/mL. To 20 µL of extract, 20 µL of porcine pancreatic amylase (0.5 mg/mL) prepared in phosphate buffer (0.02 M, pH 6.9) was added. The mixture was pre-incubated at 25 °C for 10 min before introduction of 20 µL of freshly prepared starch (1% w/v in distilled water). Tubes were incubated at 25° C for 10 min, afterward, 40 µL of DNS was added and the tubes boiled for 15 min to stop the reaction and quantify reducing sugars. 600 µL of distilled water was added to the tubes before absorbance were read at 540 nm. The control was made up of the same constituents with the extract replaced by the buffer.

Percentages of inhibition of each concentration were calculated as follow:

$$\% \text{ inhibition} = \frac{DOc - DOt}{DOc} * 100$$

Where D0c = absorbance of control and DOt = absorbance of the test.

The mechanism of alpha amylase inhibition was assessed using the same procedure as described before. The formulation was used at a concentration of 100 µg/mL with different concentrations of substrate (1.25, 2.5, 5 and 10 mg/mL). 1/V = f (1/S) (where V = velocity and S = substrate concentration) graph was plotted for determination of the mode of inhibition.

Acute toxicity

The acute toxicity of the extract was evaluated as per recommendations of OCDE, on evaluation of the acute toxicity of chemical products (23). Two groups of six female rats each, aged 8-12 weeks and weighing between 140 and 180 g, fed on normal chow and received tap water *ad libitum*. The treated group received a single dose of the okra seed extract (5000 mg /Kg of body weight) by oral gavage, while the control received a vehicle at the same dose. Animals were attentively observed for 2 h following administration of the extract, and after each six (06) h during the first day before a daily observation for14 days. Animals were sacrificed and theirs organs collected, observed, weighed and compared to those of the control.

Oral glucose tolerance test (OGTT)

After an overnight fasting (8 h), animals were given water or the extract at 200 mg / kg of body weight before receiving (5 min after) a D–glucose solution (2 g / kg of body weight). The blood glucose(expressed in mg/dL) was then measured(5–10 μL from tail tip)after 0 min (T_0), 15 min (T_1), 30 min (T_2), 60 min (T_3), 90 min (T_4) and 120 min (T_5) using a portable glucometer (Accu-Chek).

Antidiabetic effect in high fat high sucrose + streptozotocin induced diabetes

Induction of diabetes

Human type 2 close diabetes mellitus was induced on obese albinos Wistar rats by single intra peritoneal administration of 40 mg/Kg body weight of a freshly prepared streptozotocin solution. Streptozotocin was prepared in citrate buffer (0.1 M, pH 4.5). After streptozotocin administration, blood obtained from tail puncture was used to assess fasting blood glucose (FBG) of the animals 3 days later and those with a FBG$\geq$ 1.26 dg/dL were considered diabetic and used for the experiments.

Rats were divided into 4 groups of six rats each as follow:

T-: Normal control (healthy rats).
T+: Diabetic control (untreated obese and diabetic rats).
Okra: Obese and diabetic rats treated with okra seeds' extract at 250 mg/Kg of body weight once per day.
Met: Obese and diabetic rats treated with metformin at 0.25 mg/Kg body weight once per day.

Fasting blood glucose, food intake and body weight

Blood was collected by cardiac puncture for estimation of plasma biomarkers, while samples of key organs like liver, kidneys, heart, pancreas and lungs were collected for evaluation of specific markers (ALT, AST and ALP) and estimated oxidative stress markers at their levels.

Blood lipid profile and atherogenic index

Serum Triglyceride (TG), Total cholesterol (T Chol) and HDL cholesterol (HDL Chol) was determined according to the procedure describe on the commercial kit used (MONLAB). LDL cholesterol was estimated using the equation of Friedewald et al. (24):

$$\text{LDL} = \textit{total Cholesterol} - [\textit{HDL Cholesterol} + (\textit{Triglycerides}/n)]$$

$n = 2$, if results are expressed in mmol/L and $n = 5$, when results are expressed in mg/dl. The atherogenic index was calculated as follow:

$$\text{Atherogenic index} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

Renal, hepatotoxicity and oxidative stress markers

Renal and hepatoprotective activity of the okra seeds extract was assessed by evaluating key biomarkers. Alanine Amino-Transferase (ALT), Aspartate Amino-Transferase (AST) and Alcaline Phosphatase (ALP) were tested to investigate liver functions, while plasmatic and urinary creatine levels were studied to monitor kidneys functions. All measurements were made as described in the commercial kits used (Teco Diagnostics, USA).

Malondialdehyde (MDA) level and reduced glutathione (GSH) were evaluated as oxidative stress markers in the plasma and at the level of the key organs up mentioned. MDA was determined according to the method described by Yagi (25) while GSH was determined as per the method of Ellman (26).

Statistical analysis

Designing and analysis of the results were done using Minitab 18. Experiments were carried out in triplicates. Statistical significance of the variables was determined at 5% probability level. Main effects and contour plots were plotted using Sigma Plot v11.0 (c) Systat. Data on phenol and flavonoid contents, as well as those on biochemical parameters were expressed as mean± SD and analyzed by One way Analysis of variance (ANOVA) using SPSS version 22 (IBM). Comparison were made using Bonferroni test at 5% significance.

Results and discussion

Optimization of the extraction of polyphenols from okra seeds using the central composite design

Three factors were studied for extraction of polyphenols from okra seeds, namely the microwave wavelength, the time of extraction and the solvent/dry matter ratio. Ethanol proportion was decided to be 50% based on previous work. Table 2 represents the experimental and predicted response values in different variable conditions given in real and coded values.

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Analysis of main effects

The entire experimental plan consisted of 20 trials. The highest polyphenolic content (87.66 ± 3.33 mg of GAE/g) was obtained at 330 W for 9.5 min of treatment time with 97.04 mL of solvent. The lowest content (45.45 ± 2.33 mg GAE/g) was observed at 330 W of microwave power with 55.00 mL of extracting solvent and a heating time of 0.25 min. These values are greater than those of Peter et al. (27) and Hu et al. (28) who obtained a total phenolic content of 20.2 and 21.1 mg GAE/g from okra seeds by water and methanol extraction respectively. The highest phenolic content was also greater than what obtained by Geng et al. (11) from okra flowers by ethanol extraction (40.77 ± 0.83 mg GAE /g material). Such differences could be related to the extraction method. Microwaves induce a quick elevation of the temperature, thus leading to a rapid breakdown of cell walls and liberation of polyphenols out of the matrix (5).

Effect of time

The effect of time on the total phenolic content is illustrated in Figure 2. Increase in the exposition time from 2 to 9.5 min led to an increase in the phenolic content, probably due to the breakdown of cell walls under the heat generated by the microwave, thus leading to a progressive liberation of polyphenols in the solvent system. From 9.5 min, any increase in the extracting time leads to a progressive reduction in the polyphenolic content of the extract obtained. This may be the result of progressive destruction of these thermo-sensitive compounds under long exposure to heat. Previously, the similar effect has also been noticed by Sanja et al. (29) and Xuan et al. (30).

Effect of solvent ratio

An almost linear increase in the phenolic content of the extracts was observed for any increase in the solvent/dry matter ratio (Figure 2). The highest content was obtained with the ratio 97.04 mL/g thus suggesting that high solvent ratio increases mobility of compounds (mass transfer) from plant matrix to the solvent system, as already reported by (31).

Effect of wavelength

It can be seen from Figure 2 that any increase in the extracting power from 77.73 to 330 W induced an increased in the polyphenolic content of the okra seed extracts as a result of more break down of cell walls under the increased heat in the operating system alongside with the power increase, which led to more liberation of polyphenols in the solvent. But any increased in the power of the microwave apparatus above 330 W caused a progressive diminution of the polyphenolic content of the extracts as a consequence of degradation of these compounds exposed to high temperature, since usage of high power in microwave apparatus induce a quick and high elevation of the solvent temperature even when exposure is for a short duration (29).

TABLE 2 Experimental and predicted responses.

Trials	Matrix of real and coded variables			Responses	
				TPC (mg GAE/g)	
	Time (min)	Solvent (mL/g)	Power (W)	Exp	Pre
1	4.00 (−1)	30.00 (−1)	180.00 (−1)	57.27 ± 1.09	51.97
2	15.00 (+1)	30.00 (−1)	180.00 (−1)	55.27 ± 2.02	52.77
3	4.00 (−1)	80.00 (+1)	180.00 (−1)	71.31 ± 2.00	69.05
4	15.00 (+1)	80.00 (+1)	180.00 (−1)	64.65 ± 1.60	67.76
5	4.00 (−1)	30.00 (−1)	480.00 (+1)	56.69 ± 3.33	52.95
6	15.00 (+1)	30.00 (−1)	480.00 (+1)	47.65 ± 2.21	49.28
7	4.00 (−1)	80.00 (+1)	480.00 (+1)	58.84 ± 1.09	60.71
8	15.00 (+1)	80.00 (+1)	480.00 (+1)	50.28 ± 1.13	54.95
9	0.25 (−1.68)	55.00 (0)	330.00 (0)	45.45 ± 2.33	50.75
10	18.74 (+1.68)	55.00 (0)	330.00 (0)	51.00 ± 1.59	46.58
11	9.50 (0)	12.95 (−1.68)	330.00 (0)	58.24 ± 0.90	63.83
12	9.50 (0)	97.04 (+1.68)	330.00 (0)	87.66 ± 3.33	82.96
13	9.50 (0)	55.00 (0)	77.73 (−1.68)	53.22 ± 1.09	57.04
14	9.50 (0)	55.00 (0)	582.26 (+1.68)	50.03 ± 3.45	47.09
15	**9.50 (0)**	**55.00 (0)**	**330.00 (0)**	68.36 ± 1.30	68.12
16	**9.50 (0)**	**55.00 (0)**	**330.00 (0)**	68.24 ± 1.90	68.12
17	**9.50 (0)**	**55.00 (0)**	**330.00 (0)**	67.89 ± 2.00	68.12
18	**9.50 (0)**	**55.00 (0)**	**330.00 (0)**	68.54 ± 3.10	68.12
19	**9.50 (0)**	**55.00 (0)**	**330.00 (0)**	68.01 ± 2.89	68.12
20	**9.50 (0)**	**55.00 (0)**	**330.00 (0)**	67.84 ± 1.09	68.12

Exp, experimental; Pre, predicted; Bold values are replicates of the center points.

ANOVA, regression equations for the responses

Table 3 below shows the ANOVA and the influence of each independent factor. Taken individually, solvent/dry matter ratio and the power of the microwave apparatus significantly influenced ($p < 0.05$) the polyphenolic content of the extracts. The quadratic effect of the time (X_1X_1) and power of extraction (X_3X_3) significantly influenced the total phenolic content of

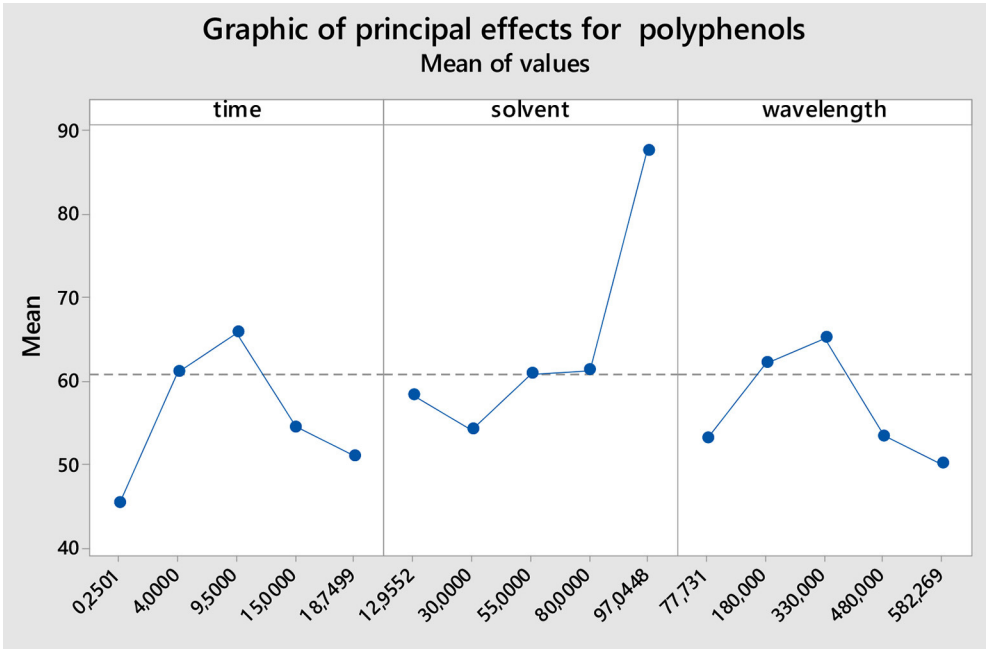

FIGURE 2
Main effect plots of individual factors on polyphenolic content.

the extracts, with the greatest contribution in the response (30.86%) for the time, followed by the quadratic effect of the microwave power (22.87%). The mathematical model predicting the effect of the factors on the response is given below (Eq. 1):

$$TPC = 7.8 + 4.75\,X_1 + 0.141\,X_2 + 0.1938\,X_3 - 0.2274\,X_1X_1 + 0.00298\,X_3X_3 - 0.000252X_2X_2 - 0.0038\,X_1X_2 - 0.00135\,X_1X_3 - 0.000621X_2X_3$$

TPC: total phenolic content; X_1: time; X_2: power; X_3: solvent/dry matter ratio.

Assessment of model quality and optimum conditions

Experimental values showed that these mathematical models can well explain the observed results. According to (32), a good mathematical model should predict at least 75% of the responses; R^2 should then range 0.75 to 1. Based on the determination coefficient for phenols (0.89) given in Table 3, it was concluded that the postulated second-order polynomial equations, truly represented the experimental data. Also, obtaining values of AADM (Analysis of the Absolute Average Deviation) and Bf (Bial factor) respectively equal to 0 and 1 confirmed the suitability of the models since values were in the normal range (0 for AADM and 0.75<Bf<1.25 for Bf).

TABLE 3 Evaluation of quadratic model: *P*-value, F value, RC, CF (Contribution Factor) (%), AADM and Bf for phenols.

Source	*p*-value	*F* value	CF (%)
Time (X_1)	0.347	0.98	1.04
Solvent (X_2)	**0.001**	20.50	21.77
Power (X_3)	**0.040**	5.55	5.89
X_1^* X_1	**0.000**	31.63	30.86
$X_2^*X_2$	0.158	2.32	4.22
X_3^* X_3	**0.001**	21.53	22.87
X_1^* X_2	0.757	0.10	0.11
X_1^* X_3	0.512	0.46	0.49
X_2^* X_3	0.186	2.01	2.14
Validation of the model			
R^2		0.89	
AADM		0.00	
Bf		1.00	

Bold, Factors that significantly ($p < 0.05$) influenced the polyphenolic content.

Optimization of the process

After validation of the model, the optimal extraction conditions for extracting phenols from okra seeds were determined using response surface curves. Figures 3A–C illustrates the variation in the polyphenolic content of okra seed extracts under the influence of different factors taken two by two, drawn with Minitab 18. These figures show that maximum content of phenolic content (87.66 ± 3.33 mg of GAE/g) is obtained at 330 W, with a solvent ratio of 97.04/1 for 9.5 min.

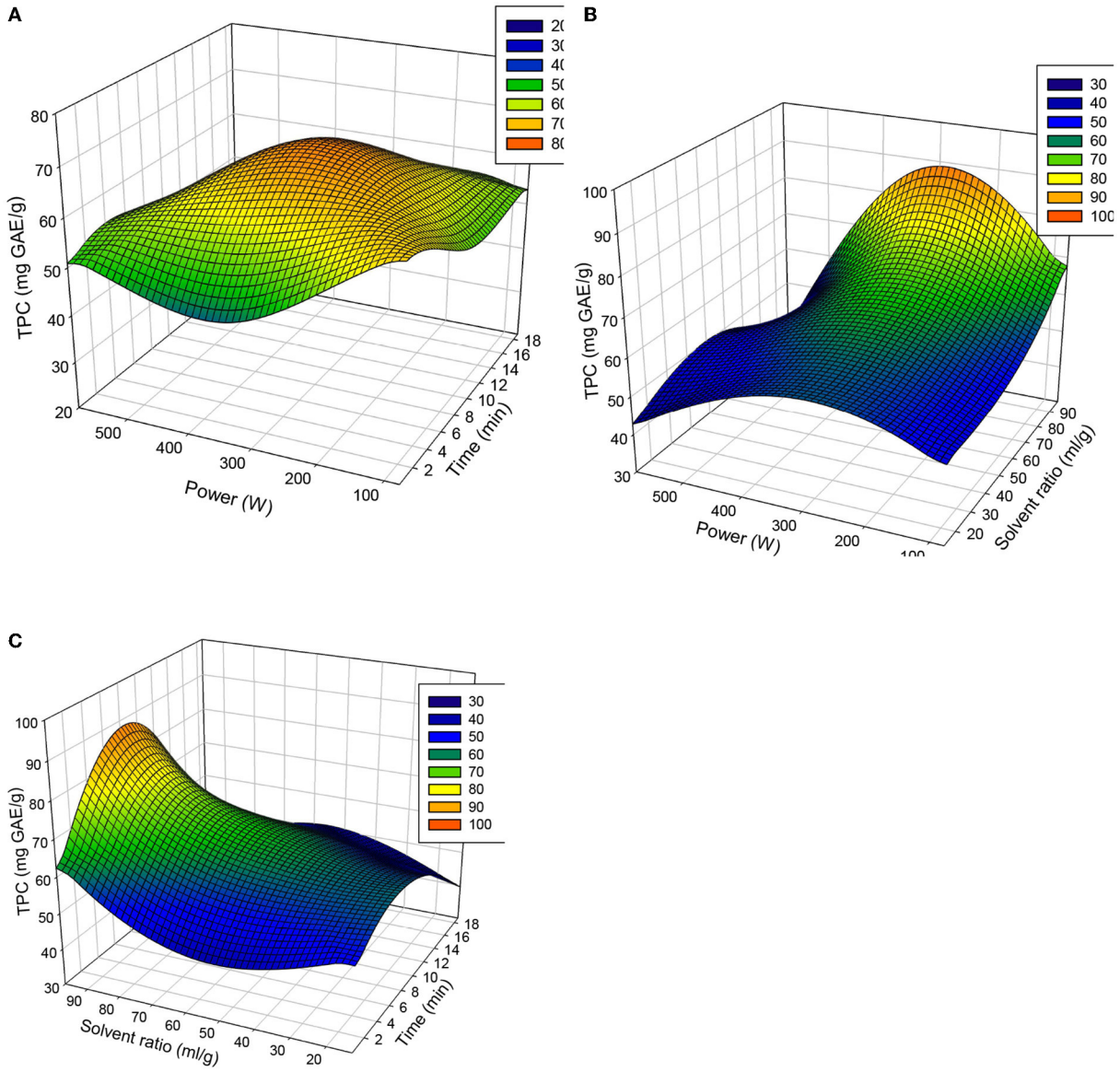

FIGURE 3
(A–C) Response-surface curves for phenolic content considering the different factors taken 2 by 2.

Confirmation experiments

Replications of the optimum conditions proposed by the model were made in order to confirm the quality of our model to predict the optimal conditions for TPC. No significant difference was noticed between optimal predicted and experimental values obtained, thus confirming the validity of the predicted optimal value given by the software as shown in Table 4. The optimization process permitted to determine the conditions of maximum extraction of polyphenols from okra seeds' as a TPC of 86.77 ± 1.52 mg GAE/g were obtained, a value two-fold greater than the maximum content of 39.39 ± 7.46 mg GAE/g obtained by Graham et al. (33) on different okra seeds cultivars.

Characterization of the extract

Phytochemical characterization of the extract

Table 5 show the phytochemical characterization of the extract obtained with the optimal conditions.

Antioxidant activity

Tables 6, 7 show the antioxidant capacities of the okra extract measured by the FRAP and DPPH scavenging methods. The extract performed better than BHT in both tests. It showed a very high DPPH scavenging ability in a concentration dependent

manner with an IC_{50} of 3.99 ± 0.15 µg/mL, at least 7 times smaller than the minimum value obtained by 32. Optimization permitted to produce high antioxidant extract compared to what have been previously reported (28, 33). The great antioxidant capacity of the extract should be related to its high phenolic content, since polyphenols have been reported to possess antioxidant capacities (33).

Thin layer chromatography

Figure 4 shows the different spots observed on TLC plate. The presence of five (05) spots with Rf ranging from 0.17 to 0.97, led to the conclusion that the optimized extract of okra seeds' contained at least 5 compounds or groups of compounds. Among these compounds, quercetin and catechin were identified with Rf of 0.94 and 0.97 respectively. These observations are in accordance with those reported by Peter et al. (27) and Ong et al. (3) who showed the presence of quercetin and its derivatives in okra seeds' extract. The Rf values of the different spots observed and a tentative identification is given in Table 8.

FTIR spectral analysis

The functional groups of the bioactive compounds present in the extract were tentatively identified using spectral analysis. Figure 5 is the FTIR spectra of the extract while Table 9 summarizes the different bands obtained and their assignation. Ten (11) bands were observed among which eight were characteristic of molecules possessing antioxidant and antidiabetic activities, including phenols and flavonoids.

Acute toxicity

No visible change on the behavior or the macroscopic aspect of the main organs of the animals, were noted after administration of the extract to normal rats up to 14 days after administration. The optimized extract of okra seeds was then said not to be toxic at unique dosage intake of up to 5000 mg/kg body weight. These results are in accordance with those of (42) who reported that okra hydroalcoholic extract did not show any toxicity or death up to a dose of 5000 mg/kg in Wistar rats. Uddin et al. (43) had also found okra mucilage powder and peel–seed mixture to be safe at a dose level of up to 1000 mg/kg of body weight in mice.

Oral glucose tolerance test

Table 10 shows the blood glucose concentrations of animals at different time during the oral glucose tolerance test. The okra seeds' extract limited the increase in the glucose level by 24.20 % compare to the normal control. Also, a quick drop down was noticed in the blood glucose level of rats treated with the optimized okra extract. This could be due the presence of soluble fibers in the extract which adsorbed glucose in the intestine, thus preventing it absorption into the blood (13).

Alpha amylase inhibitory activity and mechanism

The optimized okra seeds' extract was able to inhibit porcine pancreatic alpha amylase for up to 24.80 % at concentration of 200 µg/mL as shown in Table 11. Lineaweaver-Buck plot permitted to classify the okra extract as a non-competitive inhibitor of alpha amylase (Figure 6). Similar observations were made by Quan et al. (44) who explained that the inhibitory activity could be due to the presence of phenolic compounds in the extract. Also, a possible synergistic interaction between polyphenolic and the terpenoid compounds could justify the great alpha amylase inhibitory activity of certain extracts compared to others (44). The observed result could also be

TABLE 4 Experimental, predicted values and desirability for polyphenolic content in optimal conditions.

	Predicted value	Experimental value	Desirability
Phenol (mg GAE/g)	85.12[a]	86.77 ± 1.52[a]	0.93

On the same line, values with different letters significantly differ ($p > 0.05$).

TABLE 6 Absorbance of the at different concentration during the FRAP assay.

	Concentration (µg/mL)				
	12,5	25	50	100	200
AB	1.51 ± 0.17[b]	3.02 ± 0.06[b]	3.20 ± 0.06[b]	3.15 ± 0.01[b]	3.14 ± 0.03[a]
BHT	0.18 ± 0.02[a]	0.40 ± 0.15[a]	0.45 ± 0.09[a]	0.97 ± 0.01[a]	2.70 ± 0.10[a]

AB, *Abelmoschus esculentus* extract; BHT, Butyl hydroxyl–toluene; [a,b]in the same column, values with different letters differ significantly ($p < 0.05$).

TABLE 5 Phytochemical composition of the optimized okra extract.

Parameters	TPC (mg GAE/g)	TFC (mg CE/g	Fiber (g/100g)	Saponin (mg/g)	Proteins (mg/g)	Zinc (mg/g)
	86.77 ± 1.52	2.62 ± 0.27	1.84 ± 0.06	0.43 ± 0.05	0.175 ± 0.020	0.003 ± 0.00

TPC, total phenolic content; TFC, total flavonoid content; EC, equivalent catechin.

TABLE 7 Percentage of DPPH inhibition at different concentrations.

	Concentration (μg/mL)					
	12.5	25	50	100	200	IC_{50}
AB	50.57 ± 0.18^{b}	62.22 ± 0.04^{b}	87.18 ± 0.08^{b}	91.44 ± 0.01^{b}	91.29 ± 0.00^{b}	3.99 ± 0.15^{a}
BHT	15.84 ± 0.00^{a}	20.16 ± 0.09^{a}	24.91 ± 0.08^{a}	38.63 ± 0.22^{a}	53.31 ± 0.15^{a}	$4.4x10^{11} \pm 15.28^{b}$

AB, *Abelmoschus esculentus* (okra) extract; BHT, Butyl hydroxyl–toluene. ^{a,b}in the same column, values with different letters differ significantly ($p < 0.05$).

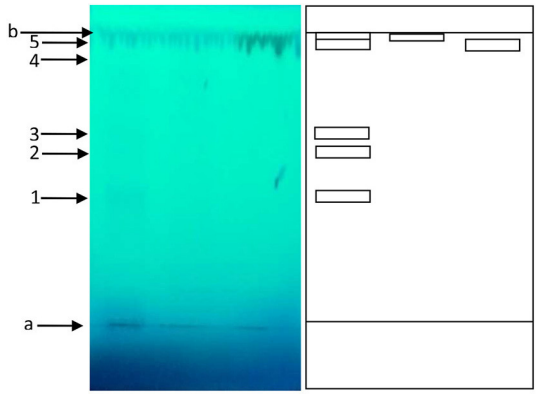

FIGURE 4
Chromatogram of okra extract under UV 254 light **(left)** and schematic representation **(right)**. a= deposit line, b= front line. 1, 2, 3, 4 and 5 are the different spots observed under UV light (254 nm).

TABLE 8 Rf and identification of the different spots obtained on TLC with okra seeds' extract.

Bands	1	2	3	4	5
Rf	0.18	0.32	0.59	0.94	0.97
Identification	/	/	/	Quercetin	Catechin

due to the fiber content of the extract since (45) and Nsor-Atindana et al. (46) previously demonstrated that cellulose in a concentration and particles size-dependent manner can inhibit alpha amylase and alpha glucosidase.

Antidiabetic activity of the optimized okra extract

Effect of the treatment on the fasting blood glucose (FBG), the body weight and the food intake

A significant decrease in the FBG was observed in the group treated with the okra extract compared to the negative control. The optimized extract exhibited better hypoglycemic activity than metformin. Such activity could be explained by the presence of polyphenols which are able to stimulate glucose absorption at the muscular level, or to increase insulin production by the pancreas. Flavonoids like quercetin that were found in the okra extract can induce the expression of the glucose transporter GLUT 4 or inhibit PPARγ 1, which end up in an increased absorption of glucose in muscles (47).

At the end of the treatment, a significant weight loss was notice in untreated diabetic rats, as a consequence of diabetes (48). The okra extract was then able to alleviate muscular weight loss in treated diabetic rats, probably by ameliorating the assimilation and utilization of glucose at the levels of cells, the weight loss in diabetics being a direct consequence of the inability of the body to use circulating glucose. Similar observations were made by Gupta et al. (49, 50), with plant extracts on diabetic rats.

A decrease of 2.57 mg in the food intake of the rats treated with the okra extract was observed, thus suggesting the extract had been able to alleviate polyphagia in animals. The observed activity can be related to the soluble fibers contained in the extract, which may limit the production of orexigenic compounds like ghrelin by enhancing the production of appetite suppressors like cholecystikinin, Glucagon-like Peptide 1 (GPL-1) and peptide YY (50–52). Table 12 summarizes the variations in the different parameters.

Effect of the treatment on the lipid profile and the atherogenic index

The treatment with okra extract significantly lowered blood triglyceride (TAG), total cholesterol (T Chol) and LDL cholesterol concentrations in diabetics rats. HDL cholesterol concentration was significantly elevated in the AB group, compared to diabetic and normal controls. The atherogenic risk was significantly reduced in okra treated diabetic controls and metformine treated rats, thus leading to the conclusion that treatment of diabetic patients with okra extract could reduce the risk of cardiovascular accidents. Presence of flavonoids like quercetin (53) or alkaloids (54) in the extract may explain these activities. Esmaeilzadeh et al. (55) reported that okra extracts could stimulate the production of alpha hydroxylase, the enzyme converting cholesterol into bile acids in the

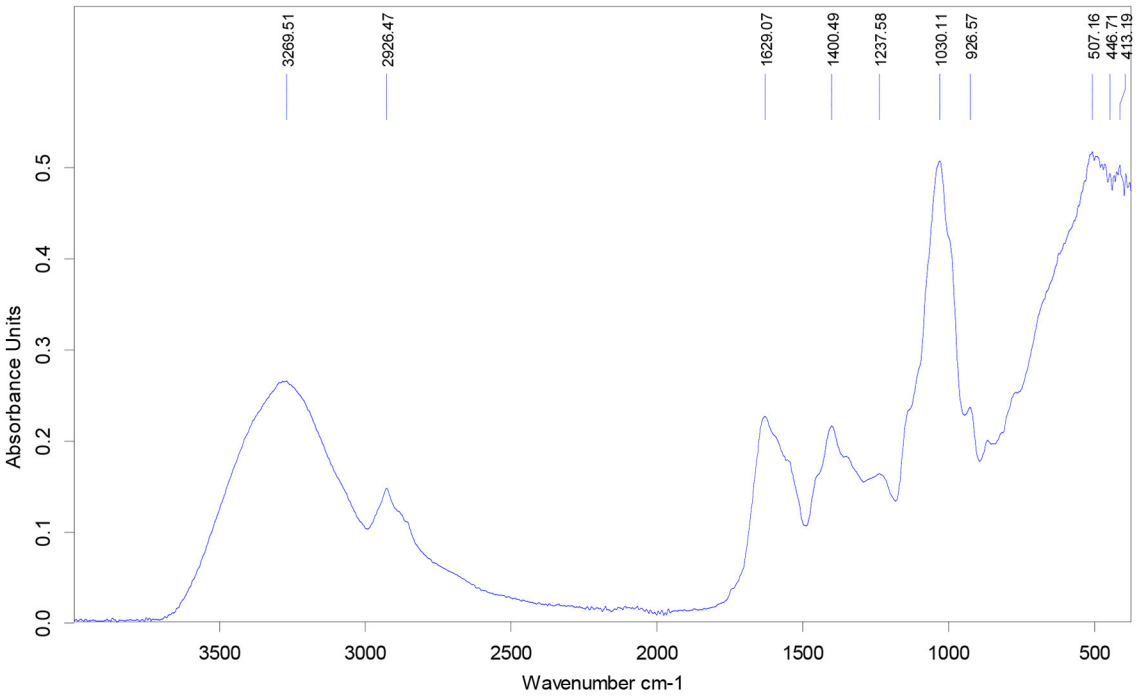

FIGURE 5
FTIR spectra of the optimized okra seeds' extract.

TABLE 9 Peak wave numbers and tentative identification.

Bands (cm^{-1})	Vibration	Assignation	Reference
3269	–C–OH (stretching)	Water, cabohydrates	(34, 35)
2926	–C–H (stretching)	Aliphatic portion of lipids	(35)
		Isoflavones	(36)
1629	–C=C		(37)
1400	–O–H (bending)	Phenols or tertiary alcohol	(38)
1237	unidentified		
1030	Ester –C–O (stretching)	Glycosidic groups	(39)
926	–C–C (stretching)	Alkane: lipids, amino acids, proteins	
507	Phenolic ring (torsion)	Phenols	(40)
446	unidentified		
413	–C–OH in plane (bending)	Phenols	(41)

TABLE 10 Blood glucose levels and increment during the OGTT.

	0 min	15 min	30 min	60 min	90 min	120 min	Increment
AB	100 ± 7.54^{b}	155.33 ± 4.5^{a}	134 ± 7^{a}	124.33 ± 2.08^{a}	113.33 ± 4.93^{a}	98 ± 8^{a}	55.33 ± 3.05^{a}
T	85 ± 4.35^{a}	158 ± 6.2^{a}	141 ± 8.12^{a}	126 ± 3.46^{a}	116.33 ± 6.65^{a}	107.33 ± 7.09^{a}	73 ± 2.64^{b}

AB, *Abelmoschus esculentus* (okra) extract; T, normal control. Increment: highest blood glucose value (at 15 min)—start value (0min); ^{a,b}in the same column, values with different letters differ significantly ($p < 0.05$).

TABLE 11 Alpha amylase inhibitory percentages and IC_{50}.

	Concentration (µg/mL)				IC_{50} (µg/mL)
	25	50	100	200	
AB	10.04 ± 0.30	10.66 ± 0.76	17.13 ± 2.16	24.80 ± 2.36	484.17 ± 2.33

AB, *Abelmoschus esculentus* (okra) seeds' extract.

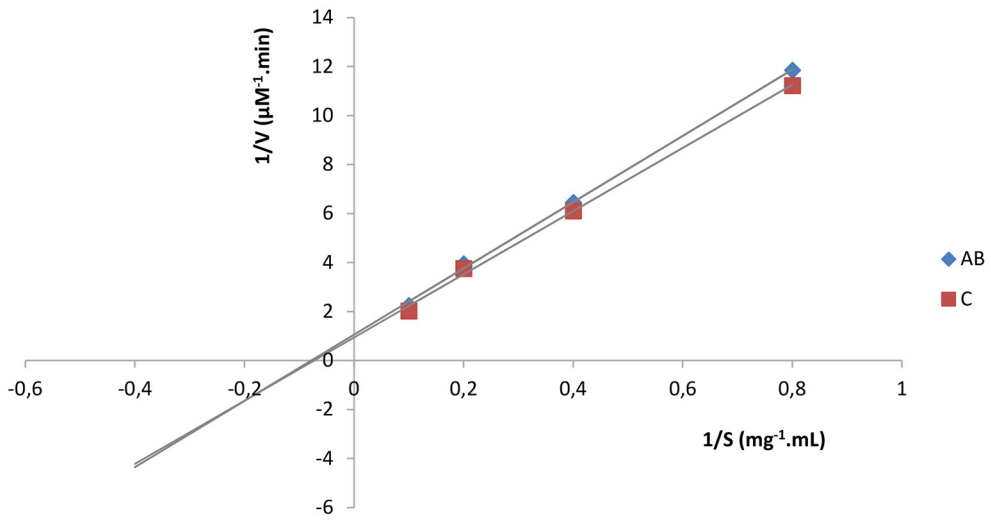

FIGURE 6
Lineaweaver-Buck plot for AB. AB, *Abelmoschus esculentus* seeds' extract; C, control.

liver, or inhibit the HMG-COA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase; resulting in a decrease in the circulating cholesterol. The extract could exert its LDL cholesterol lowering activity by limiting the intestinal absorption of lipids implicated in their formation in the liver (56). Also, Brijyog et al. (57) stated that flavonoids present in the extract could induce the transcription of lipoprotein lipase genes. The enzyme is then produced and breakdown lipoproteins like LDL cholesterol with an end result of its blood concentration reduction. Similar observations were made by Abd El Latif et al. (58) who noticed a normalization in the lipid profile of diabetic rats treated with soybean isoflavones. Table 13 gives the lipid profile and atherogenic index of the animals at the end of the treatment.

TABLE 12 Variation of some parameters between the start and the end of the experiment.

	FBG (g/L)	Body weight (g)	Food intake (g)
AB	−102 ±7.81^{a}	−2.33 ± 9.01^{b}	−2.57 ± 0.04^{a}
Met	−83.66 ± 9.23^{b}	−7.66 ± 15.94^{b}	0.32 ± 0.01^{b}
T+	26 ± 2.64^{c}	− 45.33 ± 11.06^{a}	8.46 ± 0.04^{d}
T−	2 ± 1.41^{d}	39.5 ± 8.54^{c}	4.54 ± 0.02^{c}

AB, *Abelmoschus esculentus* (okra) seeds' extract; Met, Metformine; T+, Diabetic control; T−, Negative control; FBG, Fasting blood glucose. Variation = final value−start value; a, b, c, and d, in the same column, values with different letters differ significantly ($p < 0.05$).

Renal and hepato-protective activities of the optimized okra extract

Serum Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alcaline Phosphatase (ALP) concentrations were measured to assess the integrity of the liver. The liver plays a key role in the glucose homeostasis, and diabetes could disturb its functioning, which is marked by elevated concentrations of AST, ALT and ALP in the blood, resulting from an inflammatory state induced by hyperglycemia and oxidative stress (59, 60). The okra extract exhibited hepato-protective capacities by significantly reducing these enzymes concentrations in the blood, compared to diabetic control. Similar observations were made by (58, 61, 62). The hepato-protective activity noticed with the optimized okra extract could be due to its antioxidant capacity, as (63) hypothesized that plant extracts could exert their hepato protective activity by combating the oxidative stress which is responsible of the inflammation and necrosis of the liver, resulting in the elevation of the above mentioned liver

TABLE 13 Lipid profile and atherogenic index of animals at the end of the treatment.

	TAG	T CHOL	HDL	LDL	AI
AB	33.41 ± 5.03^{a}	47.73 ± 0.23^{a}	36.73 ± 1.93^{b}	4.32 ± 0.89^{a}	1.30 ± 0.06^{a}
Met	64.81 ± 1.56^{c}	68.40 ± 2.11^{b}	36.08 ± 2.60^{b}	19.35 ± 3.69^{c}	1.90 ± 0.15^{b}
T+	42.67 ± 3.61^{b}	76.53 ± 4.73^{c}	30.94 ± 3.23^{a}	37.05 ± 5.83^{d}	2.49 ± 0.33^{c}
T–	47.43 ± 3.50^{b}	64.25 ± 4.59^{b}	38.98 ± 4.07^{b}	15.78 ± 2.31^{b}	1.65 ± 0.08^{b}

AB, *Abelmoschus esculentus*; F, Formulation; Met, Metformin; T+, Diabetic control T–, Normal control. TAG, Triglycerides; T CHOL, Total cholesterol; HDL, HDL cholesterol; LDL, LDL cholesterol; AI, Atherogenic index; a, b, c, and d, in the same column; values with different letters differ significantly ($p < 0.05$).

TABLE 14 Effect of the treatment on liver biomarkers.

	AST (UI/l)	ALT (UI/l)	ALP (UI/l)
AB	38.11 ± 6.71^{a}	8.75 ± 1.54^{a}	214.65 ± 5.18^{b}
Met	74.08 ± 5.92^{b}	21.19 ± 3.75^{b}	209.15 ± 4.85^{b}
T+	89.83 ± 5.05^{c}	28.58 ± 4.04^{c}	231.17 ± 2.59^{c}
T–	40.83 ± 6.06^{a}	22.45 ± 3.94^{b}	137.60 ± 7.78^{a}

AB, *Abelmoschus esculentus*; Met, Metformine; T+, Diabetic control; T–, Normal control; AST, Aspartate amino transferase; ALT, Alanine amino transferase; ALP, Alcaline Phosphatase; a, b, and c, in the same column; values with different letters differ significantly ($p < 0.05$).

TABLE 15 Serum and urine concentrations of creatinin of the treated rats.

	Serum	Urine
Creatinin concentrations (µmole/L)		
AB	2.53 ± 0.10^{a}	172.61 ± 1.17^{d}
Met	2.47 ± 0.30^{a}	156.11 ± 9.25^{c}
T+	2.88 ± 0^{b}	41.96 ± 2.40^{a}
T–	2.38 ± 0.10^{a}	122.69 ± 7.82^{b}

AB, *Abelmoschus esculentus*; Met, Metformin; T+, Diabetic control; T–, Normal control. a, b, c, and d: in the same column, values with different letters differ significantly ($p < 0.05$).

enzymes and precisely the ALP. Table 14 summarizes the values obtained.

Among the most severe complications of diabetes, is renal failure. A good antidiabetic management should then prevent or delay it appearance. Creatinin clearance is the commonest way to assess the renal status since a high concentration of creatinin in the blood circulation can be indicative of a renal failure. The okra extract significantly reduced the blood concentration of creatinin in comparison to diabetic control (Table 15). This was in accordance with previous works (48, 56). Danish et al. (56) reported that daily administration of *Albizzia lebbeck* stem bark extracts to diabetic rats for 45 days induced a significant decrease in the serum creatinin concentration.

Conclusion

The study aimed at evaluating the antidiabetic activity of an optimized polyphenolic rich extract obtained from okra seeds. RSM was used with MAE to determine the optimal conditions for extracting polyphenols from okra seeds. It was found out that the solvent/dry matter ratio, the power of the microwave apparatus, the quadratic effect of the time (X_1X_1) and the interaction between the solvent/dry matter ratio and the operating power (X_3X_2) significantly influenced ($p < 0.05$) the polyphenolic content of the extracts. RSM permitted to define the conditions for maximum extraction of polyphenols from okra (87.66 ± 3.33 mg of GAE/g) as: microwave power of 330 W, with a solvent ratio of 97.04/1 for 9.5 min. Optimization thus permitted to determine the conditions for extraction of at least two-fold the average maximum TPC reported up to date from okra seeds. The optimized extract exhibited powerful antioxidant capacities with an IC_{50} of 3.99 ± 0.15 µg/mL in DPPH scavenging assay. It also acted as a non-competitive inhibitor of porcine pancreatic amylase, and showed good antidiabetic capacities on streptozotocin induced diabetic rats.

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

CW and DK conceived the work, collected seeds, carried out experimentations, analyzed and interpreted data, and wrote the article. DM collected the seeds, assisted in experimentations, and read the article. HW supervised the work and read the article. All authors have approved the final article.

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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Potential applications of pulsed electric field in the fermented wine industry

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Fermented wine refers to alcoholic beverages with complex flavor substances directly produced by raw materials (fruit or rice) through microbial fermentation (yeast and bacteria). Its production steps usually include saccharification, fermentation, filtration, sterilization, aging, etc., which is a complicated and time-consuming process. Pulsed electric field (PEF) is a promising non-thermal food processing technology. Researchers have made tremendous progress in the potential application of PEF in the fermented wine industry over the past few years. The objective of this paper is to systematically review the achievements of PEF technology applied to the winemaking and aging process of fermented wine. Research on the application of PEF in fermented wine suggests that PEF treatment has the following advantages: (1) shortening the maceration time of brewing materials; (2) promoting the extraction of main functional components; (3) enhancing the color of fermented wine; (4) inactivating spoilage microorganisms; and (5) accelerating the formation of aroma substances. These are mainly related to PEF-induced electroporation of biomembranes, changes in molecular structure and the occurrence of chemical reactions. In addition, the key points of PEF treatments for fermented wine are discussed and some negative impacts and research directions are proposed.

KEYWORDS

pulsed electric field, fermented wine, mechanism, aging, application

Introduction

Fermented wine, known as original juice wine, is made of raw materials containing starch and sugar (fruit or rice) through the fermentation of yeast and bacteria to produce complex flavor substances and alcohol, such as red wine, white wine, and rice wine. The production of fermented wine is a complex and time-consuming process, including saccharification, fermentation, filtration, sterilization, and a series of continuous technological procedures. Any operation introduced in the brewing process

can affect the physicochemical properties and sensory characteristics of the finished wine (1, 2). Some freshly fermented wines are not suitable for immediate drinking due to their spicy taste, pungent smell, precarious color, and aroma. In order to improve their stability, quality and sensory characteristics, these freshly fermented wines are customarily required to undergo post-processing, i.e., aging (also known as maturation) (3). However, several disadvantages of using oak barrels, pottery or other modern containers for naturally mature fermented wines, such as red wine and rice wine, are worth investigating, including long time, large storage space, and the presence of undesirable microorganisms that cause food contamination and produce unpleasant tastes (4). Therefore, it is necessary to adopt innovative technologies to optimize or accelerate the winemaking and aging of fermented wine.

Pulsed electric field (PEF) is a promising non-thermal food processing technology, which can effectively ensure the good quality of products (5–9). Recent technological developments, especially the use of continuous processing chambers, have provided more possibilities for scaling up the application of PEF technology, which has attracted widespread attention (10–12). The potential applications of PEF in the fermented wine industry have been extensively studied worldwide (13–16). Therefore, this review aims to systematically summarize the achievements of PEF technology in the fermented wine industry, and discuss the corresponding processing principles and some negative effects, so as to provide references for future research directions.

Principles of pulsed electric field

Pulsed electric field is a promising non-thermal technology, which applies a high-intensity electric field pulse for a short time (treatment time usually in the microsecond scale) to food or raw material between two electrodes (17–19). The exponentially decaying waves and square waves are commonly used in PEF processing, and square wave pulses are considered better than the exponential decay wave pulses because the former allows the material to be treated with a sustained and constant intensity for the total duration of the pulse (5). The factors affecting the efficiency of PEF treatment include electric field intensity, pulse number, pulse shape, pulse time/length, and initial temperature of processing medium (raw material), etc. (20). Among them, electric field intensity and treatment time (t = pulse duration × pulse number) are the main processing factors, and increasing the intensity of these two parameters generally improves the processing efficiency of PEF (5).

When an external electric field is applied, the charge accumulation on the membrane surface causes an increase in the transmembrane potential on both sides of the membrane. After the critical value of transmembrane potential is exceeded, the electroporation (reversible or irreversible) will be produced in cell membranes, resulting in a sharp increase in membrane permeability (21, 22). Animal and plant cells require a lower critical electric field intensity (0.5–2 kV/cm) for electroporation because their cell sizes are larger than microbial cells (10–14 kV/cm) (23). The electroporation dramatically increases membrane permeability which may affect mass transfer or cell rupture (**Figure 1**). Therefore, PEF technology has received extensive attention and research in a variety of food processing, such as food dehydration (24), sterilization (25), promotion of extraction (26), and reduction of pesticide residues (27). In addition, the PEF treatment promotes the ionization and polarization of molecules, changes the internal molecular arrangement, improves the effective collision rate between molecules, speeds up the chemical reaction rate in dynamic equilibrium, and reduces the activation energy required for the reaction. Hence, PEF is gradually used to accelerate the oxidation, reduction, association, hydrolysis, and other reactions in food (5, 25–28), especially liquid food such as fruit juices and alcoholic beverages (29). Meanwhile, compared with other non-thermal processes, such as high hydrostatic pressure (high equipment costs and discontinuous production) (30), the PEF technology also exhibits several advantages, such as shorter processing time, lower treatment temperature, and continuous flow processing (31).

Applicability of pulsed electric field

Enhancement of primordial composition extraction

The efficient extraction of functional components from target tissues or cells is a major problem prior to fermentation. The raw materials are usually pressed and macerated for a long time to extract their active ingredients such as sugar and polyphenols in traditional brewing, thus improving the yield and quality of fermented wine (32, 33). PEF is adopted as a new way for promoting the extraction of active ingredients, shortening the immersion time, and promoting alcohol fermentation. This is mainly attributed to electroporation induced by PEF treatment (**Figure 1**), which increases the permeability of cell membrane to ions and macromolecules, thus promoting the release of intracellular substances (18). For example, polyphenols, including phenolic acids, flavanols, flavonols, anthocyanins, and stilbenes, are the most important functional compounds in winemaking, since they have multiple biological effects and contribute to the wine's distinctive color (34). Unfortunately, only 40% of anthocyanins and 20% of tannins from grape skins are transferred to wine in the case of traditional winemaking (35, 36). This relatively low extraction efficiency is the result of insufficient permeabilization of cell walls and cytoplasmic membranes. Hence, PEF treatment has great potential to promote the extraction of functional

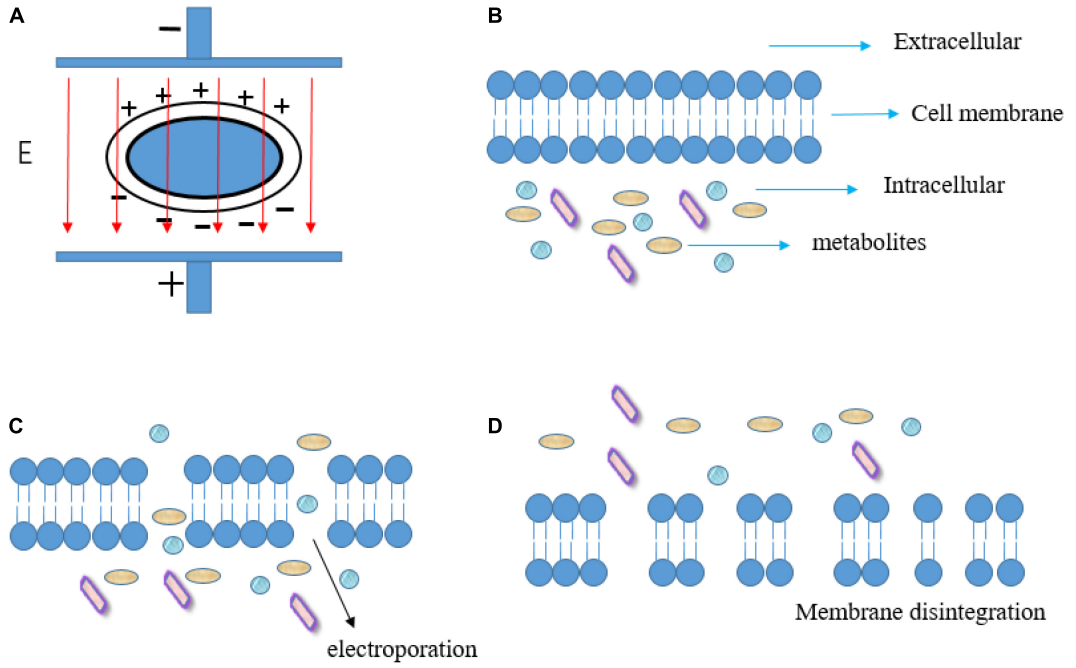

FIGURE 1
Schematic diagram of transmembrane potential induced by PEF **(A)**, membrane before electroporation **(B)**, electroporation of cell membrane **(C)**, and membrane disintegration induced by excessive PEF treatment **(D)**. Adapted from Mahesha et al. (96).

components in the maceration process. Interestingly, although PEF treatment can promote the dissolution of active ingredients during the maceration stage, there are other studies in which the PEF treatment of the samples did not affect some enological parameters of the grape juice samples (37). Clodoveo et al. (38) describe the mechanism that PEF treatment can destroy the cell membranes of grapes, and this treatment has no effect on alcohol content, total acidity, pH, volatile acidity and concentration in reducing sugar. It is worth noting that high voltage is usually required to produce electroporation, but high-intensity PEF treatment has a strong electrolytic effect, and its high electric field strength and long treatment time may lead to the degradation of some macromolecular substances.

Improvement of juice yield

The yield of fruit juice is an important index to measure the effect of maceration, and it obviously increases by PEF treatment in the brewing process. After applying 11 kJ/kg (1.5 kV/cm, 8 μs) and 22 kJ/kg (1.5 kV/cm, 16 μs) of PEF treatments, the yield of grape juice increased by 8.9 and 4.3% compared with 78.0% (w/w) of the untreated sample, respectively (14). Similarly, an increase in "Pinot Noir" grape juice yield was observed, which may be attributed to increased cell membrane permeability and accelerated mass transfer after PEF treatment (1.5 kV/cm, 1,033 pulses) (39). Meanwhile, PEF treatment (1.5 kV/cm, 1,033 pulses) of grapes increased the release rate of anthocyanins, thus achieving an adsorption-desorption equilibrium between the anthocyanins inside the skin and outside the juice within a shorter time. As a significant property, dry extract consists of fixed wine compounds, including sugars, glycerol, organic acids, phenolics, and minerals, contribute to the development of distinctive taste and in turn affect the sensory characteristics and quality of the wine (16). Compared with the control group, the dry extract content in jujube wine increased by 10% after PEF treatment (1.5 kV/cm, 50 pulses), which may be related to the improvement of mass transfer in pulp tissues, indicating that PEF pretreatment had a positive impact on jujube wine quality (**Table 1**). The increases in total acidity and phenolics could also explain the cause of the elevated dry extract contents, which was consistent with the previous study (1.5 kV/cm, 16 μs) (14). Skin maceration and maturation in wooden barrels have also been reported to improve dry extracts and obtain good mouth-feel properties, but require a long time-consuming (40).

Promoting extraction of phenolic compounds

Phenolic compounds are important components in determining the quality of fermented wine, which influence the color, mouthfeel, and aging ability of wines. The phenolic compounds in winemaking can be divided into flavonoids (12 major subclasses, mainly anthocyanidins, flavan-3-ols, flavonols, flavanones, isoflavones, and others) and non-flavonoids (mainly hydroxyl cinnamic acid derivatives) (41). The application of PEF in the maceration stage can greatly enhance the content of phenolic compounds in fermented wine. Previous studies

TABLE 1 Application examples of PEF treatment for fermented wine.

Applications	Targets of treatment	Materials	Treatment conditions	Effects	References
Enhancement of extraction	Juice yield	Garganega white grapes	PEF: 1.5 kV/cm, 8 μs, and 16 μs	The yield of grape juice increased by 8.9 and 4.3% compared with 78.0% (w/w) of the control sample, respectively.	(14)
	Dry extract	Jujube wine	PEF: 1.5 kV/cm, 50 pulses	The dry extract content increased by 10%.	(16)
	Polyphenols	Pinot Noir and Merlot must	PEF: 8 kV/cm, 344 Hz, 300 s	The content of total phenols is 2 times and 1.5 times higher than the control sample, respectively.	(42)
	Polyphenols	Jujube pulp	PEF: 1.5 kV/cm, 0.1 kHz, 10 μs	The extraction rate was 45% higher than the untreated sample.	(43)
Enhancement of color	Color intensity	Cabernet Sauvignon red wine	PEF: 5 kV/cm, 122 Hz, 50 pulses, specific energy for 3.67 kJ/kg	The color intensity increased by 31% during aging in the bottle.	(48)
	Pigment	Marechal Foch grapes	PEF: 3.3 kV/cm and 5 kV/cm for 10 s, at a frequency of 20 pulses	The share of the red parameter (*a) in wines is respectively higher by 15 and 24% than those untreated samples.	(53)
	Anthocyanins	Cabernet Sauvignon rosé wines	PEF: 5 kV/cm, 50 pulses	14 anthocyanins were increased in different degrees after 2 months of storage in the bottle.	(54)
	Proanthocyanidins	Merlot red wine	PEF: 6–24 kV/cm, 10 μs, 10 Hz, 0–300 pulses	The effect was closer to aging when the field strength is below 18 kV/cm.	(60)
Inactivation of spoilage microorganisms	*Brettanomyces bruxellensis*	Cabernet Sauvignon red wine	PEF: 50 kV/cm, 100 Hz, 78 μs	>6 log reductions	(25)
	P. pentosaceus	Tempranillo red wine	PEF: 22 kV/cm, 154 μs, specific energy for 62 kJ/kg	1.93 log reductions	(65)
	Saccharomyces bayanus	Bogazkere red wine	PEF: 31 kV/cm, 3 μs, 40 ml/min of flow rate	5.4 log reductions	(68)
	Saccharomyces cerevisiae	Chinese rice wine	PEF: 12 kV/cm, 120 μs, 35°C,	2.88 log reductions	(77)
	Acetobacter sp.	–	PEF: 20 kV/cm, 6.0 ms, with 9% ethanol	The inactivation rate could reach 5.17 log reductions, significantly higher than that cultivated without ethanol (3.22 log).	(22)
Formation of aroma substances during aging	Fatty acids	Chardonnay white wine	PEF: 6 kV/cm, 10 μs, 100 pulses	The trend of tartaric acid, oxalic acid, citric acid, and succinic acid was similar to those aging in bottles.	(10)
	Free amino acids	Shaoxing Huangjiu	PEF: 2 kV/cm, 0.5 Hz, 36 pulses	The sweet and MSG-like amino acids reached the aging level, and bitter amino acids showed a declining trend.	(82)
	Polyphenols	Grenache wines	PEF: 4 kV/cm, 100 μs, specific energy for 6.2 kJ/kg	Flavan-3-ol monomers catechin and epicatechin promoted polymerization to form tannins similar to aged wines.	(13)
	Esters	–	PEF: 20 kV/cm, 1.0 kHz, 30 μs	The activation energy of the esterification reaction of ethanol propionate was reduced by 18.9% and the product was 1.9 times higher than that untreated.	(90)
	Fusel oil	Jujube wine	PEF: 1.5 kV/cm, 0.1 kHz, 10 μs	After 140 min treatment, the methanol content decreased by 30.7% compared with the control group.	(43)

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have shown that PEF treatments can increase the contents of total phenols, total flavonoids and anthocyanins in wine using different voltages (7–8 kV) and different frequencies (178–344 Hz) (42). The PEF treatment (8 kV/cm, 344 Hz, 300 s pulses) resulted in a wine with a content of total phenols 2 times and 1.5 times higher than the control sample in the case of "Pinot Noir" and "Merlot" red wine, respectively. Apart from phenolic compounds localized in the grape skins, PEF treatment improved the extraction of phenolic compounds that primarily originate from the grape seeds such as flavanols e.g., (+)-catechin. PEF treatment (7.4 kV/cm) on crushed grape berries of Tempranillo and Grenache has also been shown effective in enhancing (+)-catechin and (−)-epicatechin concentrations in the grape juice (37). Particularly, PEF treatment can promote the release of components that are difficult to leach through normal pathways by inducing electroporation of the cell membrane. Arcena et al. (26) observed that the flavonol quercetin along with a few anthocyanins, such as delphinidin-3-O-glucoside and petunidin-3-O-glucoside, were detected exclusively in Merlot musts pre-treated with high energy treatments, which indicated that not all phenolic compounds were easy to be extracted, thus the application of PEF was needed to enhance their extractability. Notably, PEF can also significantly increase the extraction rate of phenols in other fermented wines. Xu et al. (16) took jujube pulp as the research object and carried out different PEF treatments with 10 exponential wave pulses at 1.5 kV/cm, 1 Hz to improve the extraction of phenolic compounds, especially caffeic acid, morin, and p-hydroxybenzoic acid, which significantly strengthened the floral and fruit flavor volatility of jujube wine. In a similar report by Li et al. (43), the phenolic content in the fermented jujube wine after 20 min with PEF (1.5 kV/cm, 10 μs, 0.1 kHz) increased by 45% compared with the control group.

The variation trend of phenolic content was not consistent with different PEF treatment conditions, which is closely related to the variety of raw materials. El Darra et al. (44) applied PEF treatments with medium and high field intensity (0.8 and 5 kV/cm) to improve the total polyphenols content of the pre-treated extract of Cabernet Sauvignon red grapes during low-temperature maceration, showing that color intensity, anthocyanins content, and the extraction kinetics of phenolic compounds were significantly enhanced. Other authors confirmed that the extraction rate of phenols from Cabernet Franc grapes increased by 51 and 62% after PEF treatment (0.8–5 kV/cm, 42–53 kJ/kg), respectively (45). A similar finding was reported by investigating the effect of PEF at different electric field strengths (up to 41.5 kV/cm) and energy inputs (up to 49.4 kJ/kg) on the volatilities and phenolic profiles of "Merlot" grapes at different stages of winemaking (26). PEF treatment increased the contents of anthocyanins, catechin, stilbenes, hydroxycinnamic acid, and hydroxybenzoic acid in the wine after alcoholic and malolactic fermentation. Compared to the untreated group, juice from PEF-treated grapes was found to be at least 7–49 times higher in individual anthocyanins. The stilbenoids, flavonols, and hydroxycinnamic acids at all applied PEF intensities also increased 2–5 times, 2–11 times, and 4–6 times, respectively (26). Interestingly, the content of phenols did not necessarily increase with increasing applied electric field strength. The effect of PEF (0.9–3 kV/cm) on the extractability of polyphenols in early-harvested Sangiovese red grapes from Emilia Romagna (Italy) was investigated by Ricci et al. (46). The results showed that the extraction rate of polyphenols in the PEF-treated groups (10.4, 23.8, 32.5 kJ/kg) increased by 22.9, 16.1, and 20.4%, respectively, compared with the control group. More notably, the total phenolic content of the PEF-treated groups (10.4, 23.8, 32.5 kJ/kg) was significantly increased by 49.0, 60.8, and 60.7%, respectively, after maceration (46). Therefore, when applying a PEF treatment, the transmembrane transport of bioactive compounds was regulated by pore dimensions produced during electroporation and by the size of the passing molecules, which means that the extent of PEF intensity may modulate the composition and amount of the polyphenol components (14).

In general, according to published research results, it is not difficult to find that PEF treatment increases the content of total phenolic compounds and effectively promotes the dissolution of components, and the main influencing factors are electric field intensity, treatment time, and raw material type. Due to the electroporation effect, there are three main trends in the pretreatment of raw materials by PEF. Firstly, the yield of juice during the maceration stage was increased. Secondly, the extraction efficiency of compounds from the skins and the seed of raw materials was improved, which was manifested by the increase of phenolic substances such as hydroxycinnamic acids, malvidin-3-O-glucoside and (+)-catechin. Finally, the higher applied electric field strength may lead to a decrease in the extraction rate of polyphenols, and there is an optimal value of PEF treatment conditions.

Decreasing maceration time

The extraction of pigment compounds from the cells of the skin during the maceration step is controlled by diffusion through the cell membranes in the winemaking process (46). The extraction requires that the pigment compounds leave both the membrane-bound vacuole and the cell itself, which is a slow process that requires several days or even a dozen days, while PEF treatment can significantly shorten the maceration time and ensure adequate pigment extraction. The influence of PEF treatment (5 kV/cm, 2.1 kJ/kg) on the grape pomace on quality parameters and anthocyanins content of Cabernet Sauvignon wines obtained after different maceration times has been investigated (47). Regardless of the maceration time, the freshly red wine was richer in color intensity, total polyphenols index, and tannins, and showed better visual characteristics with PEF treatment. Meanwhile, it was detected that the concentrations of malvidin-3-glucoside and malvidin-3-glucoside acetate, as

the main anthocyanins in red wine, were higher than those in control wine. Similar results were observed in the study of Puértolas et al. (48), where the maceration time for PEF-wine was only 96 h, 33% less than the control wine that was macerated for 144 h. According to the results obtained, the application of PEF treatment can significantly reduce the maceration time during winemaking. Interestingly, when using a pre-fermentative maceration at 12°C for 6 h, PEF-wine (0.8 and 5 kV/cm) presented an anthocyanin concentration of 36% higher than the control (**Figure 2C**). In contrast, Ducasse et al. (49) using a maceration scheme consisting of 12 h at a temperature of 15°C, obtained an increment of 13% on the anthocyanin content of Monastrell rosé wines using an enzymatic preparation. This indicated that PEF treatment can effectively improve the extraction rate of anthocyanin than enzyme treatment.

Enhancement of color

The color of fermented wine is usually an important index to measure its quality. For red wine, its color is generally determined by a mixture of pigment compounds (tannins, anthocyanins, flavonols, etc.), influenced by various factors such as variety, harvest year, grape ripeness, health, winemaking techniques, age, and storage conditions (50, 51). The color was determined using the CIELab method in the produced wines, obtaining L, * a, and * b values, which are the axes of a three-dimensional color space (52). According to research, PEF has a positive effect on the color of red wine, without affecting wine characteristics such as alcohol content, total acidity, pH, sugar concentration, and volatile acidity. As reported in Ilona et al. (53), in the maceration stage, the grapes were treated with a PEF treatment of 3.3 and 5 kV/cm for 10 s (**Figure 2A**). The share of the red parameter (*a) in wines is respectively higher by 15 and 24% than those of the samples without additional pretreatment (**Table 1**).

The color of the Garnacha must after maceration significantly enhanced with the increase of PEF treatment conditions (50 exponentially decaying pulses; 1–7 kV/cm, 0.4–4.1 kJ/kg) (**Figure 2B**). With PEF treatment (5 kV/cm, 122 Hz, 50 pulses) during aging in the bottle, the color intensity and Folin–Ciocalteu index in red wine from Cabernet Sauvignon grapes increased by 31 and 25%, respectively (48). Anthocyanin content in Cabernet Sauvignon rosé wines obtained from grapes treated with PEF (5 kV/cm, 50 pulses) was investigated. After 2 months of storage in bottles, varying extents of increases were observed in fourteen different anthocyanins (unacylated, acylated, and coumarylated) compared to the control group (54). The results showed that the extraction rate of wine-specific pigment compounds was higher under the support of PEF due to the effect of electroporation, which was beneficial because the color of darker wine indicated high levels of colored antioxidant beneficial compounds. Furthermore, the application of PEF on a semi-industrial scale has also achieved satisfactory results. The researchers examined the effect of PEF pretreatment (0.4–7 kV/cm, 100 μs–10 ms) on wine color at a scale of 2 tons per hour (55). Red wine produced by PEF-treated grapes had a 20–30% higher color intensity and a 7–17% higher total polyphenol index than the control group. Surprisingly, the total anthocyanin content of PEF wine was 34% higher than that of the control.

The purplish tones of young red wine typically develop into more stable brick-like colors of matured wines during storage (56–58). During the aging period after fermentation, the proanthocyanidins in wine will self-polymerize and form high molecular weight polymers with anthocyanins. In addition, the flavan-3-ols component unit of proanthocyanidins will form condensates with anthocyanins or acetaldehyde, resulting in the maturation of wine color (59). The content of proanthocyanidins, average degree of polymerization and component units of proanthocyanidins in Merlot red wine changed significantly after high voltage PEF treatment (6–24 kV/cm, 10 μs, 10 Hz, 0–300 pulses), and the trend of change was consistent with the natural aging effect (60). When the electric field strength was lower than 18 kV/cm, the treatment effect was closer to aging with the increase of the field strength. However, it is worth noting that when the field strength was up to 24 kV/cm, the excessive field strength would promote the participation of proanthocyanidin in the chemical reaction, and its degradation rate would exceed the formation rate, leading to the decrease of treatment effect. Therefore, moderate PEF treatment is beneficial to accelerate wine aging and stabilize wine color.

Inactivation of spoilage microorganisms

Yeasts and bacteria are common spoilage microorganisms in fermented wine, which negatively affect the quality and shelf life of wine and cause detrimental economic losses (61, 62). SO_2 is generally used in the traditional winemaking process as an antioxidant and selective antibacterial additive to inhibit the growth of molds in the must during the early stage of wine production and the growth of undesirable bacteria and yeast during the fermentation process, to avoid microbial spoilage in the wine production and storage process (63). The problem is that SO_2 can cause some adverse effects on consumers including allergic reactions, headaches, asthma, and abdominal pain. Therefore, the addition of SO_2 is limited to a maximum of 150–350 mg/L. PEF leads to membrane electroporation and even disintegration due to its short and high electric field intensity pulses (**Figure 3**), which have a significant inactivation effect on microorganisms. Hence, the application of PEF to the microbial inactivation step of fermented wine can reduce or even replace

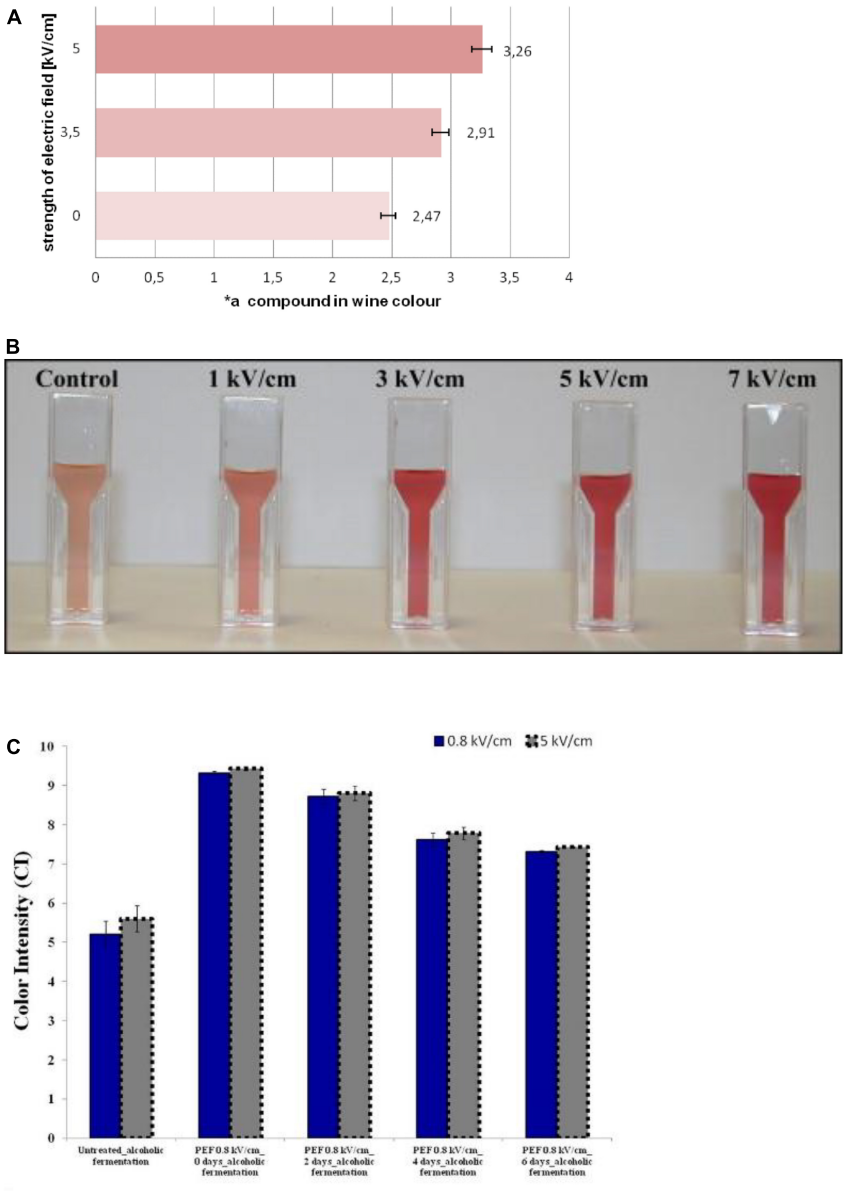

FIGURE 2
(A) Share of the red component (*a) in the color of the control group and PEF treatment (3.3 and 5 kV/cm) wines. Reprinted from Puértolas et al. (48). **(B)** The color of the Garnacha must significantly enhanced with the increase of PEF treatment (1–7 kV/cm, 0.4–4.1 kJ/kg, 50 pulses). Reprinted from Hui et al. (85). **(C)** Color intensity (CI) at t = 0, 2, 4, and 6 days of Alcoholic Fermentation using both high (5 kV/cm) and moderate (0.8 kV/cm) PEF. Reprinted from El Darra et al. (44).

the amount of SO_2 addition in winemaking, while achieving the expected sterilization effect (64, 65).

Lethal effects

Pulsed electric field treatment has proven to be a highly efficient wine pasteurization technique because it can inactivate key spoilage microorganisms in a short time, while retaining the distinctive flavors and aromas of the wines produced, without influencing taste and quality (66). There may be a variety of spoilage yeasts in winemaking, such as *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Zygosaccharomyces fermentati*, and species of *Candida*, *Pichia*, and *Hansenula*, which may lead to the formation of thin-film growth on the surface of the wine, affecting the quality of wine (67) (pp. 535–676). A variety of bacteria can also be present in wine, such as *Lactobacillus brevis*, *Oenococcus oeni*, *Lactobacillus buchneri*, and *Pediococcus*, which can cause spoilage, pH rise, turbidity, discoloration, stale taste, and sediment formation (67) (pp. 535–676).

The inactivation effect is closely related to the species of microorganisms, due to the different sensitivity of

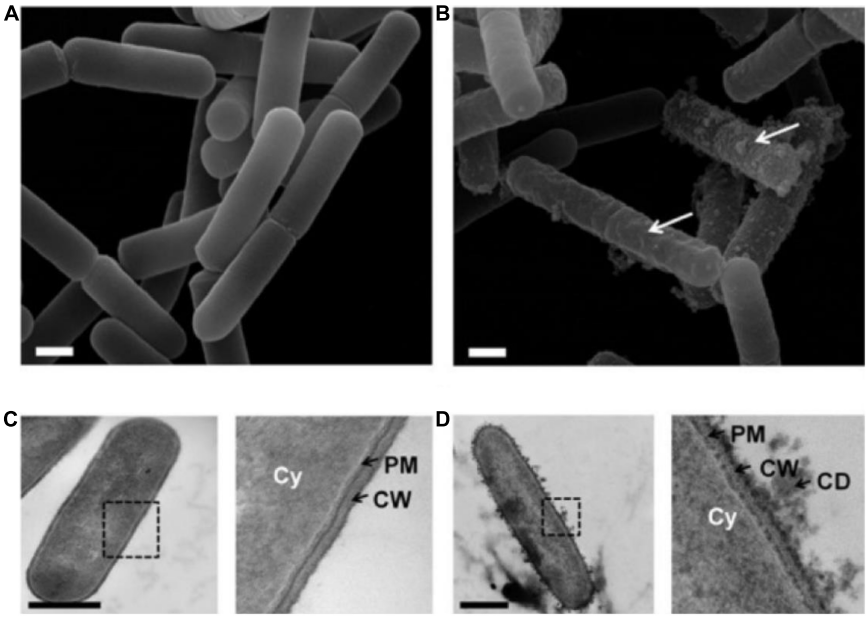

FIGURE 3
(A) SEM image of untreated bacteria. **(B)** After exposure to PEF (7.5 kV/cm, 1 ms), the SEM image showed bacterial surface damage. **(C)** TEM image of untreated *Bacillus pumilus*. Cy, cytoplasm; PM, plasma membrane; CW, cell wall. **(D)** TEM image of *Bacillus pumilus* after PEF. The illustration revealed the discharge of cell debris (CD) and the damage to PM and CW. Scale bars, 500 nm. Reprinted from Davaux et al. (71).

microorganisms to PEF treatment. *S. cerevisiae* can cause a secondary fermentation of the wine when residual sugar is present, eventually forming a precipitate in the wine. Abca and Evrendilek (68) found that a 3 μs square bipolar pulse with a field intensity of 31 kV/cm resulted in 4.5 log reductions of *S. cerevisiae* in red wine. When the same electric field intensity was applied to *S. bayanus* in red wine, the inactivation rate was significantly increased, reaching 5.4 log reductions. The PEF resistance of different wine spoilage microorganisms such as *Dekkera anomala, Dekkera bruxellensis, Lactobacillus hilgardii*, and *Lactobacillus plantarum* both must and wine was investigated by applying treatments ranging from 16 to 31 kV/cm and from 10 to 350 kJ/kg at 24°C (69). The optimal treatment with a specific energy of 186 kJ/kg has been established which permitted to reduce the 99.9% of the spoilage flora of must and wine at the field strength of 29 kV/cm, limiting the risk of alteration of these products by microorganisms of genera *Brettanomyces* and *Lactobacillus*. Concerning bacteria in wine, Gonzalez-Arenzana et al. (65) treated 12 lactic acid bacteria and 4 acetic acid bacteria in Tempranillo red wine under four different PEF conditions with specific energies of 60, 62, 72, and 95 kJ/kg, respectively. The inactivation rate was around 1.93 log reductions (*Pediococcus pentosaceus*) and 3.56 log reductions (*Acetobacter pasteurianus*) at PEF treatment of 62 kJ/kg (22 kV/cm, 154 μs), (**Table 1**). The inactivation cycles obtained with the 72 kJ/kg PEF treatment (27 kV/cm, 123 μs), were between 0.64 log reductions (*Acetobacter aceti*) and 2.44 log reductions (*Metschnikowia pulcherrima*). The 95 kJ/kg PEF treatment (33 kV/cm, 105 μs) managed inactivations between 1.96 log reductions (*O. oeni* strain O46) and 4.94 log reductions (*A. pasteurianus*).

A similar effect was achieved with PEF treatment in rice wine. Lyu et al. (77) found that 2.88 log reductions of *Saccharomyces cerevisiae* were inactivated when PEF treatment with moderate conditions (35°C, 12 kV/cm, 120 μs) was applied to Chinese rice wine. Further, Huang et al. (70) used PEF technology to evaluate the inactivation effect of *S. cerevisiae* at the initial temperature of 25–35°C, electric field intensity of 12–21 kV/cm and treatment time of 30–180 μs. The highest inactivation values, corresponding to approximately 5.5 log reductions, were obtained at 21 kV/cm and 180 μs with the initial treatment temperatures of 35°C. Davaux et al. (71) investigated the effects of the PEF (7 kV/cm) on the microbiological stability of wines on a semi-industrial scale from 500 to 1,200 L/h. When the temperature of wine without adding SO_2 did not exceed 50°C, PEF had a significant inactivation effect on yeast in wine (71). The results obtained showed an excellent efficiency of the yeast treatment with an instant cessation of alcoholic fermentation and a decrease in the yeast population ranging from 3 to 5 log reduction.

Sublethal effects

Recently, the sublethal effects of PEF on microorganisms have attracted more and more attention. There are many factors affecting the microbial inactivation by PEF and one of the key contributing factors is critical electric field intensity,

which is defined as the minimum electric field intensity required for microbial inactivation. Previous studies have shown that the intensity of the applied electric field must exceed a critical electric field intensity for electroporation (reversible or irreversible) of the cell membrane to occur (72, 73). Notably, when the cell membrane undergoes reversible electroporation after PEF treatment, a sublethal state of the cells may be induced (73). In general, the critical electric field strength value depends on microbial characteristics, especially on cell size and shape, and the characteristics of the growing medium (72). Critical electric field intensity was lowest for the yeast (14.83 kV/cm) and it was 18.64 kV/cm for the aerobic bacteria (73). Whereas, the highest critical electric field intensity value of 19.46 kV/cm was noted for the lactic acid bacteria (73). This variation in PEF sensitivity of the microorganisms could be due to the differences in cell size since the relatively large-sized cells (yeast) inactivated at the lowest electric field intensity. Generally, with the increase of voltage amplitude, capacitance capacity, and discharge times, the bactericidal rate keeps improving. When the injection energy is more than 12 J/ml at normal temperature, the bactericidal rate of PEF (15–25 kV/cm) is more than 97%, and the maximum 2-log reduction inactivation rate can be achieved (74).

Brettanomyces bruxellensis is mainly found in barrel-aged red wines with low SO_2 content and high pH (75). The researchers inoculated the wine with *B. bruxellensis* and then treated it with continuous PEF. The concentration of *B. bruxellensis* in wine was determined after inoculation, ranging from 2.3×10^5 to 5.9×10^5 CFU/ml. The *B. bruxellensis* concentration decreased to 9.2×10^4 CFU/ml (0.8 log reduction) after PEF treatment (32 kV/cm, 250 Hz, 51.2 μs). However, the yeast concentration had returned to the same level as the untreated wine after 2 months. Therefore, moderate PEF treatment conditions which could have induced a sublethal state instead of cell death, are not sufficient to inactivate *B. bruxellensis* to produce microbiologically stable wines (66). To completely inactivate *B. bruxellensis* in wine, more demanding PEF conditions are required, including higher electric field intensities and longer treatment times. A recent study found that multiple PEF treatments (50 kV/cm, 100 Hz, 78 μs) achieved the expected level of *B. bruxellensis* inactivation in Cabernet Sauvignon red wine (>6 log reductions) (25).

It is worth noting that the efficiency of PEF inactivating microorganisms is also closely related to food medium (processed raw materials) factors such as alcohol and temperature. For example, *Acetobacter* sp. is also one of the spoilage microorganisms during winemaking, converting ethanol produced by yeast into acetic acid, which increases the volatile acidity of the wine. The effect of ethanol as a growth substrate on PEF resistance in *Acetobacter* sp. cells was investigated by Niu et al. (22). The inactivation rate of *Acetobacter* sp. (10^9 CFU/ml) cultivated with 9% ethanol by PEF treatment (20.0 kV/cm, 6.0 ms) could reach 5.17 log reductions, which was significantly higher than that cultivated without ethanol (3.22 log). According to the report, the combination of eugenol and PEF has a strong synergistic bactericidal effect. The inactivation level of *Escherichia coli* was increased from 0.39 to 0.96 log when the added eugenol concentration was increased from 0 to 0.64 mg/ml and PEF treatment intensity was 20.0 kV/cm (76). Besides, increasing the initial temperature of fermented wine during PEF treatment had a significant effect on microbial inactivation. The survival yeast decreased with 4.09 log reductions when the Chinese rice wine was preheated to 35°C and followed by PEF treatment (18 kV/cm, 150 μs). In comparison, the yeast inactivation was 2.05 log reductions at an initial temperature of 25°C and the same PEF treatment (77).

In summary, PEF used for microbial inactivation can maximize the sterilization effect, inactivate enzymes, protect the nutritional components in wine, and require low processing temperature, a short processing time of several decades microsecond to sterilize. However, it was very difficult to summarize and systematically classify resistance to PEF based on size, shape or membrane structure due to the high number of different microorganisms. The inactivation of microorganisms associated with fermented wine depends on the PEF conditions applied and the characteristics of the microorganisms (78). The diversity of microbial species which are PEF resistant indicates PEF sterilization needs strict verification before application.

Formation of aroma substances during aging

Freshly fermented wines usually have some disadvantages such as sourness, thin taste, stimulating taste, and many other negative impacts due to the high content of fusel oil, acetal, and free alcohol. Hence, aging is needed to stabilize the wine quality, while various biological and chemical reactions occur during the aging process, resulting in complex aromas and full-bodied mouthfeel (79). It is reported that high-intensity PEF treatment (usually >15 kV/cm) can accelerate the chemical reaction rate in dynamic equilibrium and reduce the activation energy required for the reactions (**Figures 4**, **5**), which can significantly promote the formation of various aroma components such as thiol, acids, polyphenols, and esters in fermented wine (28). For example, it was found that Merlot red wine vinified with PEF-treated grapes possessed higher thiol concentrations and higher intensities of blackcurrant flavor character at different electric field strengths (up to 41.5 kV/cm) and energy inputs (up to 49.4 kJ/kg) (26).

Fatty acids

Moderate PEF markedly increased the fatty acid content of fermented wine because it enhanced the oxidation of ethanol to aldehydes and further to acids (80). Different moderate PEF treatments (6–24 kV/cm) and pulse numbers (100–300 pulses)

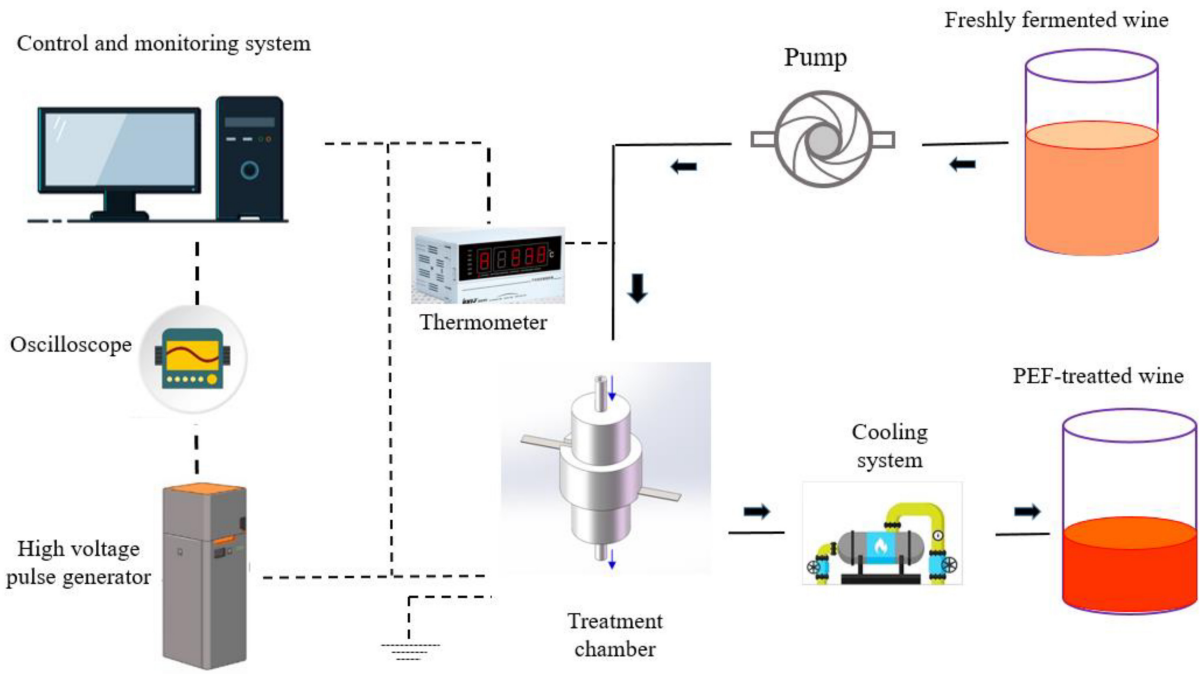

FIGURE 4
Schematics diagram of a PEF treatment system for fermented wines. Adapted from Wang et al. (10).

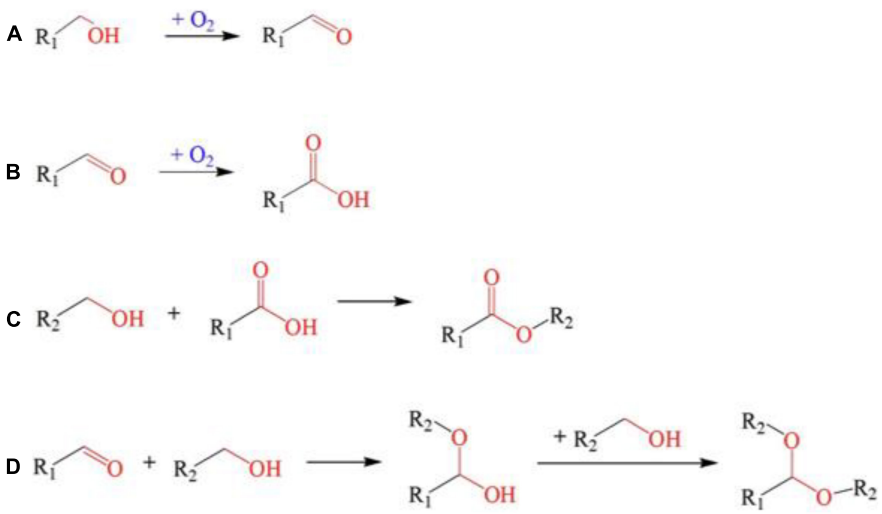

FIGURE 5
The reactions promoted by PEF treatment. **(A)** Alcohols oxidized into aldehydes; **(B)** aldehydes oxidized to acids; **(C)** Esterification reaction; **(D)** Polymerization reaction of alcohols and aldehydes could condensate into acetal. Reprinted from Ma et al. (97).

with red wine resulted in the evolution of L-malic acid, citric acid, and succinic acid concentrations following similar trends to bottle aging (10). The variation trends of citric acid, oxalic acid, and succinic acid in naturally aged white wines, were similar to those of white wines treated by PEF (6 kV/cm, 100 pulses) (**Table 1**). The amount of L-malic acid in the wine treated by PEF of 6 kV/cm and 100 pulse numbers was near to that of bottle storage for 90 days. The phenomenon revealed that the application of external PEF affected the molecular structure and chemical reactions among the substances in wine (81). Similarly, the fatty acids such as isovaleric acid, hexanoic acid, and octanoic acid in Chinese rice wine treated with PEF (2 kV/cm, 0.5 Hz, 36 pulses) could reach the level of natural aging for 3 years (82).

It is noteworthy that the variation trend of different fatty acids after PEF treatment was significantly different. For example, PEF was able to decline the content of tartaric acid in white wine and then decreased more steeply with a further increment of PEF strength. This trend is consistent with the change in bottle storage and may be related to chemical reactions between tartaric acid and other components. High levels of PEF allow the rapid evolution of tartaric acids, forming caffeoyltartaric acid, and ρ-coumaroyl tartaric acid (10). Furthermore, high-intensity PEF may have some negative effects on the changes of fatty acids in fermented wine. Succinic acid, for example, is created as a by-product of the sucrose fermentation process in wine and enables the taster to perceive the acidity, bitterness and acidity of wine in high concentrations (83). Zeng et al. (81) reported that the succinic acid content was lower than that of the untreated group due to the conversion of succinate to ethyl succinate after PEF treatment at a lower intensity. However, high-intensity PEF treatment at 18 and 24 kV/cm significantly promoted the hydrolysis of diethyl succinate, and the content of succinic acid increased (10). Therefore, the change in fatty acids depends on the strength of PEF, and there may be an optimal treatment condition for different acids.

Free amino acids

The taste characteristics of free amino acids can be divided into several categories, which were MSG-like (Asp and Glu), sweet (Ala, Gly, Ser, and Thr), and bitter (Arg, His, Ile, Leu, Met, Phe, and Val), based on the study of Azevedo et al. (84). The sweet and MSG-like components of Shaoxing Huangjiu (Chinese rice wine) treated with PEF (2 kV/cm, 0.5 Hz, 36 pulses) could reach the level of aging for 3 years (82). In terms of bitter amino acids, the contents of Huangjiu treated with accelerated aging showed a general trend of decline (**Table 1**). Compared with the naturally aged Huangjiu, PEF treatment reduced the bitterness of the wine and tempered the natural flavor of Huangjiu. This result was consistent with the research of Xu et al. (16), which found that Asp, Glu, Gly, and Ser increased after PEF treatment (1.5 kV/cm, 1 Hz, 50 pulses) in jujube wine. Free amino acids are precursors of flavor compounds (11), these free amino acids may be highly correlated to the complex synthesis of flavor compounds, which is of great significance to the overall aroma of Chinese rice wine. It is worth noting that high-intensity PEF treatment may promote the Maillard reaction between sugar and amino acids in rice wine, thus reducing the content of bitter-type amino acids (44, 84, 85).

Polyphenols

The content of polyphenols in fermented wine after PEF treatment was increased, such as flavan-3-ols, flavonols, hydroxycinnamic acids, and their derivatives, which is due to the high concentration of precursors in PEF-wine (86). For example, high concentrations of tartrate esters are hydrolyzed into their corresponding free acids such as caffeic acid and coumaric acids, leading to an increase in hydroxyl cinnamic acid. The surge in hydroxybenzoic acids, particularly gallic acid, protocatechuic acid, and syringic acid, may have resulted from the cleavage of anthocyanins during wine storage (e.g., malvidin 3-O-glucoside may lead to the production of syringic acid) (87). However, PEF treatment may also lead to a reduction in the evolution of some specific phenolic substances during aging. For red wines treated with PEF at specific energy (49.40 kJ/kg), prolonged storage at 4°C significantly reduced the amount of 11 Phenolic compounds, including anthocyanins, flavonols, and stilbenes (80). As mentioned earlier, this reduction may be due to degradation, oxidation, and cleavage auto association, as well as co-pigmentation, polymerization, and formation of new pigments (88). Due to the high initial anthocyanin content throughout the winemaking process, the level is still above that of naturally aged wines, despite the loss of large amounts of anthocyanin during storage. Moreover, the condensation reaction between (+)-catechin and acetaldehyde was enhanced by the PEF treatment (**Figure 5**). With the increase of PEF intensity ranging from 0 to 50 kV/cm, the decrease rate of (+)-catechin increased obviously. It was reported that at 40 kV/cm, the content of (+)-catechin after 31.12 ms reaction was roughly equivalent to the control group for 62.23 ms reaction without PEF treatment. Meanwhile, PEF treatment could significantly reduce the activation energy of the condensation reaction from 41.59 to 28.98 kJ/mol (89). Similarly, polyphenol flavan-3-ol monomers catechin and epicatechin promoted polymerization to form tannins under PEF treatment (4 kV/cm, 100 μs, 6.2 kJ/kg), which was similar to naturally aged Grenache wines (13).

Esters composition

Esters are the main source of aroma in wine (90) and PEF treatment can promote the esterification reaction in fermented wine (**Table 1**). The reaction rate and product concentration of the esterification reaction increase with the increase of electric field intensity. The effect of PEF (0–35 kV/cm, 1.0 kHz, 30 μs) on ethanol esterification of propionate was investigated by Lin et al. (91), while ethyl propionate had a distinctive fruity aroma. The content of ethyl propionate produced by PEF treatment was 1.30, 1.80, and 1.9 times higher than that without PEF treatment when the reaction temperature was 20°C and the field strength was 6.6, 13.3, and 20 kV/cm, respectively. Similarly, ethyl lactate possessed a distinctive rum, fruit and cream aroma. When 20 kV/cm PEF was applied and the reaction temperature was 25, 30, and 35°C, the yield of ethyl lactate was 1.7, 1.8, and 1.4 times of the sample without PEF (92). In addition, PEF can reduce the activation energy of esterification under certain conditions. When the electric field intensity is 13.3 kV/cm, the activation energy of esterification is 62.85 kJ/mol, which is 18.4% lower than the 77.05 kJ/mol of the control sample. In practical

application, Shen et al. (82) treated Chinese rice wine samples with PEF (2 kV/cm, 0.5 Hz, 36 pulses) to explore the effect of PEF on the aroma substances of rice wine. The oxidation and esterification reaction could be promoted and the ethanol content would be greatly reduced in the PEF-treated Chinese rice wine. Fatty acid ethyl esters treated with PEF (2 kV/cm) could reach the level of natural aging for 3 years, especially in medium-chain (C6–C12) and long-chain (C13–C18) fatty acid ethyl esters. Among them, ethyl 2-hydroxypropionate, ethyl butyrate, ethyl valerate, and ethyl palmitate increased most significantly (82). The technical principle of PEF promoting aging is to use the energy provided by high-voltage to change the wine body into a strong oxidation state and accelerate a series of reaction processes such as oxidation-reduction and esterification (**Figure 5**).

Unpleasant substances

Pulsed electric field treatment can reduce the concentration of some irritant volatile substances below the reported odor threshold, potentially having a positive effect on overall wine perception. The fatty acids such as octanoic acid, capric acid, butyric acid, and 3-methylbutyric acid significantly decreased in the presence of PEF (11, 22 kJ/kg) (14). The concentrations of the last three compounds were lower than the odor threshold (15, 10, and 3 mg/L, respectively) after PEF treatment, which could cause a cheese-like, rancid and pungent odor at high concentrations (93). In addition to fatty acids, the concentrations of some volatile phenols (such as 4-vinylphenol and 4-vinylguaiacol) were also significantly reduced by PEF processing. The presence of these two compounds in white wine and red wine is due to enzymatic decarboxylation of cinnamic acids by yeasts and is generally considered a defect in wine because they have unpleasant smells similar to drugs and paints (94). Most notably, excessive fusel oils which are a mixture of higher alcohols in freshly wine will make wine bitter, spicy, and rough taste. Appropriate high voltage PEF treatment (5–20 kV/cm) can promote the oxidation of fusel oil in wine, so that it can transform into aldehydes or acids, resulting in a decrease and corresponding reduction of fresh wine irritation (95). Similarly, some researchers pretreated jujube pulp with PEF (1.5 kV/cm, 10 μs, 0.1 kHz) and found that within a certain range, along with the increase of PEF treatment time, the methanol content in the fermented jujube wine decreased significantly, and the maximum reduction reached 30.7% compared with the control group after 140 min of treatment (43).

Challenges and future trends

In previous studies, the potential of PEF in the raw material extraction, sterilization and aging of fermented wine has been extensively explored in laboratory studies, and gradually applied in pilot-scale and semi-industrial production. Nevertheless, further investigations should be carried out for some controversial results about the application of PEF in fermented wine.

For commercial application, PEF needs to achieve breakthroughs in the core components of equipment such as high-power supply and intelligent control systems. The focus should be on the applicability of PEF for broad-spectrum processing of different materials such as high conductivity or high viscosity, the uniformity under the action of the physical field, and the controllability of the processing temperature. At the same time, the stability, processing capacity and production efficiency of PEF system should be greatly improved to realize the large-scale application, continuous processing and intelligent control of PEF in the fermented wine industry. Moreover, since the electrode is in direct contact with the wine, the electrochemical reaction at the interface may cause corrosion and migration of the electrode material, resulting in the flavor of the PEF-treated wine being affected. Some researchers even tasted metallic in some PEF-treated samples (15). Therefore, it is necessary to develop more suitable or more durable electrode materials to replace the commonly used stainless steel electrode.

On the other hand, the principle of accelerated aging of fermented wine after PEF treatment and the optimal application parameters of PEF are still unclear. The complex reactions of multiple compounds occur under the action of PEF, and flavor in wine is affected by the combination of all the compounds, which means that some trace substances may have a unique effect on flavor. It is difficult to capture the effects of trace substances in conventional analysis methods, making it difficult for researchers to detect subtle changes caused by PEF. More importantly, for specific compounds, such as phenols, alcohols, acids, esters, and other aroma components, the effect of PEF is not all positive. The effects of PEF on some desired substances have disparate trends for different fermented wines. However, most current research on PEF mainly focus on the application of pre-maceration and the evolution of phenolic components during red wine aging while other kinds of fermented wine are rarely reported. Furthermore, various aging conditions such as container material and aging temperature, give the wine a kaleidoscope of styles during aging. PEF treatment alone cannot completely achieve the complex aroma and taste of the natural aging process. Therefore, combining PEF technology with other processing technologies such as ultrasound, microwave, and micro-oxygenation to obtain fermented wines that are closer to natural aging may be a direction worth exploring.

Conclusion

Research on the applicability of PEF in the fermented wine industry suggests that PEF can not only shorten the maceration

time of brewing raw materials and promote the extraction of main functional components, but also enhance the color of fermented wines, inactivate spoilage microorganisms, and accelerate the formation of aroma substances during the aging process. Additionally, appropriate PEF treatment can reduce the levels of some unpleasant substances, especially fusel oil in freshly fermented wines. Furthermore, some laboratory and semi-industrial studies on the application of PEF technology have achieved the expected results. However, it is worth noting that there are still some bottlenecks that need to be solved urgently, such as the development of corrosion-resistant electrodes, high-power supplies, and intelligent control systems, which hinder the large-scale industrial application of PEF technology in the fermented wine industry.

Author contributions

YF: writing—original draft, software, formal analysis, and visualization. TY: writing—review and editing and validation. YZ: conceptualization and data curation. AZ: investigation and resources. LG: writing—review and editing and investigation. DN: funding acquisition, supervision, writing—review and editing, resources, and project administration. 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 insight into bisphenol A, food exposure and its adverse effects on health: A review

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Bisphenol A (BPA) is a synthetic chemical widely employed to synthesize epoxy resins, polymer materials, and polycarbonate plastics. BPA is abundant in the environment, i.e., in food containers, water bottles, thermal papers, toys, medical devices, etc., and is incorporated into soil/water through leaching. Being a potent endocrine disrupter, and has the potential to alter several body mechanisms. Studies confirmed its anti-androgen action and estrogen-like effects, which impart many negative health impacts, especially on the immune system, neuroendocrine process, and reproductive mechanism. Moreover, it can also induce mutagenesis and carcinogenesis, as per recent scientific research. This review focuses on BPA's presence and concentrations in different environments, food sources and the basic mechanisms of BPA-induced toxicity and health disruptions. It is a unique review of its type because it focuses on the association of cancer, hormonal disruption, immunosuppression, and infertility with BPA. These issues are widespread today, and BPA significantly contributes to their incidence because of its wide usage in daily life utensils and other accessories. The review also discusses researched-based measures to cope with the toxic chemical.

KEYWORDS

bisphenol A, endocrine disruptor, synthetic chemical, polycarbonate plastics, epoxy resins, BPA toxicity

Abbreviations: BW, bodyweight; CAGR, compound annual growth rate; CEF, EFSA panel on food contact materials, enzymes, flavorings, and processing aids; EFSA, European food safety authority; EPA, environmental protection agency; FAO, food and agriculture organization; FDA, food and drug administration; FSH, follicle-stimulating hormone; $GABA_A$, aminobutyric acid type A; IGF-1, insulin-like growth factor 1; PC, polycarbonate; PK, pharmacokinetic; PVC, polyvinylchloride; RAC, risk assessment committee; SCENIHR, EU scientific committee on emerging and newly identified health risks; TDI, tolerable daily intake; USD, United States dollar; WHO, World Health Organization.

Introduction

Xenoestrogens or endocrine disruptors are natural or synthetic compounds harmful to the endocrine system because they stop endogenous hormone production and normal functioning (1). Due to rapid advancement in human lifestyle, endocrine-disrupting chemicals are being introduced competently into the environment extensively, and living beings are directly or indirectly exposed to harmful chemicals such as Bisphenol A (BPA) (2–4).

Bisphenol A is among toxic chemicals, first highlighted by Aleksandr Dianin in 1891, then in 1905, they were made by Zincke using acetone condensation with two correspondents of phenol. In the mid-twentieth century (1940), a sudden rise in polymers (polycarbonates, polysulfone, polyacrylate, and epoxy resins) was observed with BPA. The polymers were also used as an antioxidant and endpoint for inhibiting polymerization in polyvinyl chloride plastics. Besides, flame retardant polymers, including tetrabromobisphenol-A were also prepared using the polymers (5). It makes different polymers, such as epoxy resins, polycarbonates, and other polymer materials (6, 7). Epoxy resins and polycarbonates were in high demand in 2015, 64 and 34%, respectively. Demand increases are expected with each passing year (8).

Additionally, in recent years, their uses have expanded to produce optical and electronic materials. Polymers also produce plastic food containers, drinking glasses, bowls, cups, and microwave-safe utensils (9, 10). Canned materials can be a significant source for food adulteration owing to direct contact since epoxy resins are utilized to protect the can from the inside (11, 12). They are used in other industries like the ink and paint industry, manufacturing of thermal papers, compact discs, electronics etc. (12, 13).

Since then, BPA has been abundantly used in food packaging materials, take-away water bottles, and lacquer coatings for tin cans causing human exposure to BPA *via* food and drinks (2–4). Furthermore, occupational workers get BPA exposure through direct contact with skin or inhalation, whereas the standard population is exposed to BPA by dust inhalation (14, 15). BPA has been linked to several serious health issues in animal model research. Numerous human-based epidemiological and observational studies on BPA exposure revealed similar results. BPA exposure is associated with the incidence of growth disruption, halting normal development, infertility, endocrine system disruption, immune system suppression, and carcinogenicity (16, 17).

By keeping in view the scenario mentioned above of BPA production and its utilization in different domains of life, the present review is aimed to elaborate on the different human BPA exposure routes and adverse health effects of toxicity with a special focus on basic mechanisms of endocrine disruption, infertility, carcinogenesis, and immunosuppression.

Global production

The estimated global volume of BPA utilization was 7.69 million metric tons in 2015 for different applications that were forecasted to increase to 7.7 million in 2016. Approximately 4.8% compound annual growth rate (CAGR) is observed from 2016 to 2022. The production is predicted to increase to 10.7 million metric tons in 2020 because of the broad applications of polycarbonate plastics and epoxy resins in every field. The global demand for BPA is estimated to reach USD 22.49 billion in 2022. It was recorded to be approximately USD 15.6 billion in 2015, with a forecast of 16.4 as of 2016, marking a faster CAGR of 5.4% in value. The largest market of BPA is located in the Asia Pacific, contributing to approximately 52% of the market share, while 36% is produced by the USA and Western Europe (18, 19). Due to these applications, its extensive scale application is observed in everyday life, such as in producing papers, toys, water pipes, electronic products, and other plastic materials (20, 21).

Physico-chemical properties

The molecular weight of BPA [4, 4-isopropylidenediphenol; 2, 2-bis (4-hydroxyphenyl)-propane] is 228.29 g/cm^3, with a white crystal-like appearance and highly reactive due to the presence of hydroxyl group in the structure. The melting and boiling points of the toxic chemical are 156 and 220°C (at 5 hPa), respectively. The coefficient of BPA in water octanol is expressed in a logarithmic form value of 3.32 (log P = 3.32), indicating its high solubility in fats and less soluble in water (about 200 mg/dm^3 at 25°C). Moreover, it can also be transformed into the ether, esters, and salts like other phenols. Additionally, the electrophilic substitution of BPA generally includes sulphonation, alkylation, and nitration (20, 22).

Applications of bisphenol A

Bisphenol A is a well-known synthetic chemical globally used to manufacture different polymers, including epoxy resins, polycarbonates, and other polymer materials. Polycarbonates and epoxy resins are prominent polymers in significant bisphenol applications. Some other uses of bisphenol A include the production of different resins (unsaturated polyester, polysulfone, polyetherimide, and polyacrylate) (6, 7). In 2015, global demand for polycarbonates and epoxy resins was 64 and 34%, respectively. Moreover, the rise in demand for these two polymers will be observed with an average annual rate of 3 and 4% for the next 5 years (8). Furthermore, in recent years, their applications extended to manufacturing optical and electronic materials. Plastic cups, bottles, bowls, food containers, and utensils used for microwaves are also synthesized with polymers (9, 10).

Epoxy resins protect the can from the inside; therefore, they can be a considerable source for the adulteration of food items due to direct contact (11, 12). The storage bottles are also layered with epoxy resins for a similar purpose (23). Nonetheless, epoxy resins are also successfully applied in the paint and ink industry. Beyond this, epoxy resins also have a well-established reputation in manufacturing thermal paper, compact discs (CD), and digital video discs (12, 13). Whereas the derivate compounds from BPA are used in tickets and newspapers for antioxidants and stabilizers (15, 24) and, in the textiles industry, it is employed for infant socks preparation (25).

Exposure to bisphenol A

Bisphenol A is present almost everywhere in our surroundings and significantly affects our life. It can be part of the food and environment directly or indirectly, affecting living organisms.

Environment

Bisphenol A is an 'omnipresent' contaminant due to its presence in all possible resources that might be the source of its human exposure through air, water, and soil (26). There are three main routes for human exposure environmental, occupational, and contaminated food consumption (27). Workers synthesizing BPA and their related derivative compounds (i.e., polycarbonate, epoxy resins, and polyvinyl chloride) are easy targets for occupational exposure. The main reason for the environmental exposure is the contamination of the atmosphere, soil, and aquatic systems owing to the BPA entering the environment due to its use in thermal paper recycling and relevant industries (27, 28).

According to the findings of Zhang et al. (29), who assessed the BPA concentration in water of different areas of China, 19 out of 20 water treatment plants had 5–14 ng/L of BPA. A similar situation was reported in Canada, France and South Germany. The main reason behind the increased incidence of BPA is the increased occurrence of epoxy resins and polycarbonate plastics. According to the Global Bisphenol A (BPA) Market Report and Forecast 2021–2026 report, the worldwide BPA market was $10.92 billion in 2020. The expected Compound Annual Growth Rate (CAGR) is 7.8% between 2021 and 2026 (30). Manufacturing of BPA products, their utilization, aging, and disposal in the environment are the major reasons behind the addition of BPA in ecosystems (31). Point sources include effluents from sewage treatment facilities and landfill leachate, whereas non-point sources include epoxy resin and polycarbonate plastic shards that infiltrate aquatic bodies (32).

According to various studies, approximately 56 μg/L of BPA can be ingested from the aquatic environment, 1–150 μg/kg from soil (33, 34), while 2–208 ng/m^3 of BPA can be inhaled from the surroundings and dust contributes 0.2–17.6 μg/g contamination (35). In addition, contaminated seafood ingestion, metallic food cans, and plastic bottles can contribute 13.3–213 μg/kg, 2–82 ng/g, and 0.234 μg/L, respectively (36), whereas landfill leachates (17.2 mg/L) (37), dermal route (7.1–71 μg/day) (38), and dental material (0.013–30 mg/day) also contaminate the environment (39).

Food

Food exposure is the most important because fulfilling daily dietary needs is essential for survival (**Table 1**). Contamination of BPA through food exposure occurs due to the use of BPA for manufacturing different types of plastic containers [polycarbonate (PC) and polyvinylchloride (PVC) plastics] used for food serving and exposing their direct interaction with food. Epoxy resins are also used to manufacture food cans for inner coatings. Therefore, canned food products also play a significant role in adulterating food items. Residual monomers of these compounds migrate from the can to the food product, and food consumption causes safety issues in individuals (11, 12). Besides, food packaging materials are the primary cause of BPA accumulation in human beings. It is due to the penetration of BPA from packaging into foodstuff and beverages (40, 41). There are also secondary reasons which lead to exposure to BPA and hence the infected human population (25, 42, 43).

Migration of bisphenol A particles into the food system

The migration of particles from the wrapping material to the food material is quite a complex phenomenon. It depends on different factors, including the composition of different food items, duration of contact time, the food temperature during contact, and packaging material type refs. Studies revealed that fat in foods also contributes to the migration of particles. Similarly, there is also a direct relationship between the square root of contact time and the concentration of molecules being migrated. Moreover, the high-temperature also leads to a rapid migration rate of residues (11, 12). BPA penetration into packaged products accelerates at higher temperatures (used for boiling water) than the lower temperature, around 20°C. It is also illustrated that the migration rate could be 55 folds more than the latter temperature (44). Food present in packaged products shows less absorption of BPA than the food preserved

TABLE 1 Level of BPA (g/kg) in different food commodities.

Food	BPA level	References
Cereals	0.9–3.7	(118)
Fish	7.2–103	(119)
Fruits and vegetables	10.99–94	(120)
Canned (fruits and vegetables)	3.6–267	(121)
Canned soft drinks	0.033–3.9	(122)
Milk	1.33–175	(123)

in canned materials with a standard concentration of 0.45 ng per 100 g (45).

Moreover, compounds like epoxy resins and PVC are used in manufacturing industries to protect the inner side of the can from corrosion and rust development due to direct contact with different food items (23). Bottles used for storage purposes also have such types of protective glaze. Monomers residues of BPA migrate into the food during high-temperature processing and storage in these bottles due to incomplete polymerization (23, 46).

Babies fed on mother milk or non-PC bottles had minimum BPA levels compared to babies using PC-free packaging. BPA levels were low compared to infants' body mass using non-PC feeders and eating solid foods (6–36 months) (47). The research was conducted in two steps to check BPA levels in fresh, frozen, and canned foodstuff (using 204 samples). Firstly, they checked the BPA concentrations in canned products and then calculated BPA intake through diet. Results showed that the foods not packed in canned material had lower BPA levels (7%) than canned foods (73%), while dietary assessment of adults revealed canned coatings materials as the main BPA contributor. Altogether 12.6 ng/kg was calculated per day in the human body, out of which 12.4 ng/kg was penetrated from canned foods. Moreover, 3–12.95 ng/kg per day was the tendency of dietary consumption. In contrast, 30–70 ng/kg/day was determined in the urinary bladder, higher than the central capacity of dietary intake (48).

Human exposure to bisphenol A

Bisphenol A is almost everywhere in our environment and is released from common consumer goods (**Figure 1**). It may enter our body through different routes like dermal and oral exposure or only through inhalation (49). The primary route of exposure is dietary exposure, including consumption of seafood or even freshwater fish polluted *via* BPA, fresh food commodities from polluted regions, ingestion of food packed in plastic and cans containers, and drinking polluted water (50). The second foremost route of absorption for BPA is dermal exposure (51). Direct paper contact (especially thermal paper), toys, and medical devices proportionally increase the BPA potential against the skin. Inhalation is the third most important route of exposure through BPA-containing vapors, mists, dust, and gases (50).

Bisphenol A -human exposure's primary source is canned food items. Therefore, this compound's exposure mainly depends on the duration and amount of canned food usage in a person's diet regimen. In kids older than 3 years, BPA exposure's highest mean value was approximately 69.9 ng/kg body weight/day, with an utmost value of 189 ng/kg body weight/day. In adults, 139.9 ng/kg body weight/day was the highest mean value, with the maximum contact up to 419 ng/kg

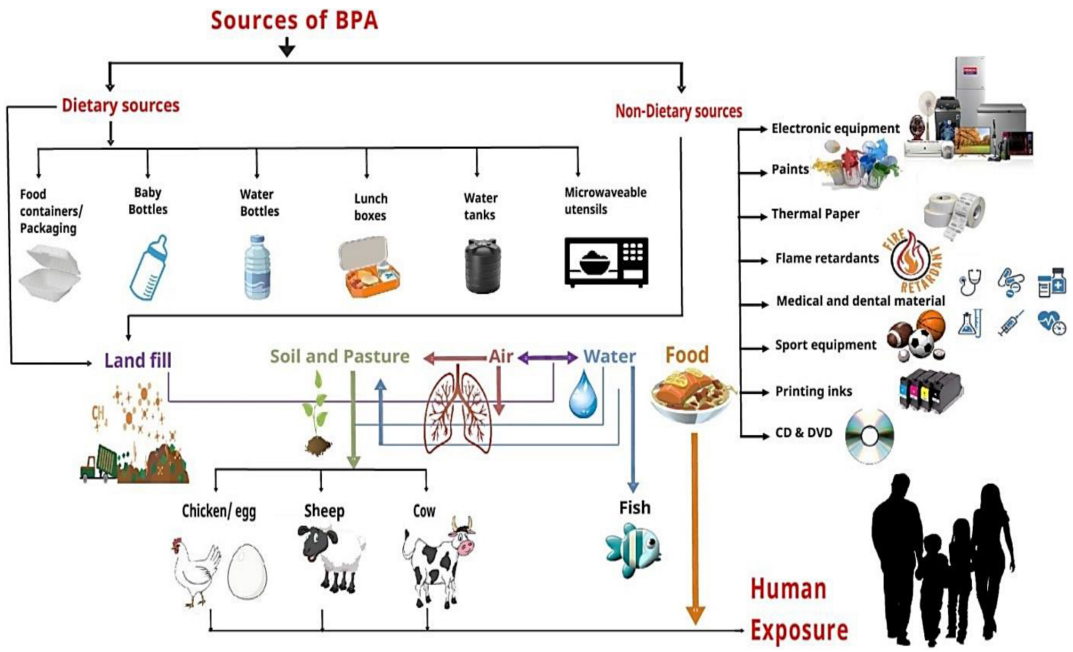

FIGURE 1
Humans exposure to BPA *via* different sources and exposed roots. Dietary sources (food containers/packaging, baby bottles, water bottles, lunch boxes, water tanks, and microwave utensils) and non-dietary sources (electronic equipment, paints, thermal paper, flame retardants, medical and dental materials, sports equipment, printing inks, and DVDs) contaminate landfill, soil, air, water, and food that directly or indirectly affect the human through different exposure routes.

body weight/day (47). Wilson and colleagues estimated in a study that the exposure through inhalation for toddlers (1.5–5 years) was 0.23–0.42 ng/kg of body weight per day (52).

Generally, infants (0–6 months old) are the most affected through the alimentary canal all over the population with BPA exposure and its disruptions. The main reason for this exposure is the daily feeding of canned milk formulas in plastic feeder bottles containing PC. Hence, these plastic feeding bottles and canned milk powders make them most vulnerable to BPA's side effects. Various research studies have been conducted to asses BPA levels and found that the BPA concentration is less than tolerable daily intake (TDI) (47).

Although, BPA exposure through inhalation and dermal routes accounts for less than 5% of all contact sources. The occupational population shares a more significant proportion (50). In a study, 154 composite samples were assessed for BPA analysis and 55 samples with BPA levels ranging from 0.19 to105.0 ng/g, respectively. The experimental results also indicated that canned foods had higher BPA concentrations than other food samples (23).

Metabolism of bisphenol A

Bisphenol A is highly metabolized and secreted into urine, primarily as a glucuronide conjugate with a half-life of 2 h (53). BPA's half-life also depends on the glucuronidase enzyme, which activates BPA through deconjugation in the bloodstream, and other organs (54). Furthermore, the valid biomarker of BPA exposure is total BPA (including free or conjugated) urinary concentration (55). The health aspect of free BPA (a weak estrogen) has been primarily observed in animal models. At the same time, limited neonatal human researches are available to check behavioral and executive functional effects, especially during critical child developmental stages and in the shortening of congenital space in male offspring (56, 57). However, several experiments revealed that BPA initially affects hepatic injury (53, 58). After ingestion, most BPA in the liver and gut is rapidly bound with glucuronic acid to release BPA glucuronide (BPA-G) by the glucuronidation process, facilitated by many enzymes (59).

Moreover, being fat-soluble, BPA has high adipose tissue affinity and is then released steadily to other histological structures in humans and mice (60, 61). An investigation to estimate the BPA division in humans highlighted that BPA is demonstrable in almost all human histological structures. In adipose tissues, it ranged from 1.13 to 12.27 ng/g, 0.78 to 3.34 ng/g in the liver, and 1 to 2.35 ng/g in the brain. In breast milk, total BPA was observed as 1.09 ng/mL, out of which 0.41 ng/mL content was identified as unconjugated BPA (6).

Furthermore, the conjugated BPA does not combine with the estrogen receptor (ER); therefore, they are biologically inactive and inert. However, another investigation revealed that BPA-conjugated forms could disturb cellular responsive action throughout membrane ERα contacts, which is responsible for quick signaling feedback (62). In contrast, in trace concentrations, unconjugated BPA (free BPA) can convert into other compounds such as BPA sulfate or BPA-S.

Bio-monitoring records reveal that BPA interaction with humans is prevalent (55, 63). However, there is still massive controversy on the legality of the reported measure of unconjugated BPA in whole blood, plasma, or serum. The discussion point is that adult human blood samples have up to 0.5–2 ng/mL (2.2–8.8 nM) unconjugated BPA. It is very high than the predicted levels of 0.51 μg/kg of body weight per day calculated based on adults' estimated daily intake (48). Presumably, few of the even most substantial daily consumption records in this range (and lower) depend on the whole day urinary output, with back calculations of 596 German women and men (64).

Pharmacokinetics of bisphenol A

According to the research conducted by Volkel et al., subjects were given a dose of 54–90 μg/kg of BW/day orally. The results showed that unconjugated BPA was not recognized in urine or serum in any human oral pharmacokinetic (PK) study. Though, the maximum value of finding in the study, 2.27 ng/ml (9.9 nM), is not as much susceptible as more current competencies of 0.05–0.3 ng/ml (0.25–1.76 nM) (65–67).

Another study, utilizing a LOD range of 0.01–0.95 ng/mL, was conducted in which 10 men were given a soup having an unconjugated d6-BPA of 0.097 ng/ml (0.42 nM) at 1.5 h, followed by administration of 29.9 μg/kg of body weight of BPA (68). The d6-BPA was 0.29% of the total BPA, leading researchers to conclude that the sublingual dietary exposure and absorption were reportedly different. Studies regarding the BPA's pharmacokinetics in rats, mice, and rhesus monkeys using isotope compounds showed that oral consumption of 75 to over 1,000 μg/kg of body weight per day is essential concentrations of unconjugated BPA reported in humans (66, 69–71).

Overall, these changes led to the estimation that unconjugated BPA in the blood is linked with the preparation of the sample, storage, systematic procedure, and exposure conditions. For example, in hospitals where patients may be interacted with BPA from medical equipment or in professional settings (63, 72–78).

Impact of bisphenol A on health

In animal model studies, BPA has reportedly caused many critical health conditions. Several human-based epidemiological and observational researches showed similar findings on exposure to BPA (47). Disrupted growth susceptibility is higher

at certain phases of the life cycle on BPA exposure, halting normal development. Fetal or postnatal development stages are more critical as the body systems are not fully developed. BPA affected growth disruption due to its metabolism and elimination through enzyme systems amalgamation (16, 17). Because of the previous studies, this review will cover the effect of BPA on the human reproductive system, endocrine system, immune system, and carcinogenicity.

Endocrine disruption

The endocrine system is one of the most synchronized and complex systems. BPA is an adverse endocrine-disrupting chemical (EDC) which suppresses or alters hormonal and enzyme synthesis, secretion, release, and transportation. BPA hinders the system's activity by replacing endogenous hormones with transporter proteins (**Figure 2**). This alteration changes the free and bound hormonal concentrations present in plasma. This chemical also influences the neuroendocrine function, causing a physiological interruption in the organs. Studies have shown the increased serum level of estradiol in females and reduced testosterone in males due to BPA (79, 80).

Mental health is highly influenced by the disrupter, which causes sex-specific mental impairment and behavioral changes. Disturbed and depressive tendencies rose because "Dehydroepiandrosterone (DHEA)," a neuroactive steroid in males, is decreased, resulting in a possible pathway of the depressive-like phenotype (81). Previous studies regarding the endocrine disruption of BPA are compiled to assess its potent role (**Table 2**).

Reproductive system

Evidential studies have indicated that the reproductive system's higher interruption susceptibility is observed due to this BPA (**Table 3**). Being an easy target, the reproductive system undergoes disturbing sex hormone activity and exertion. BPA also distracts the function and primary development of the reproductive system (**Figure 3**). Recent studies reported the BPA linkage with increased levels of serum luteinizing hormone (LH), estradiol (E2), progesterone, and testosterone (T) while decreased concentrations of serum cortisol (80). A significant association between BPA and higher total testosterone (TT) concentration in serum was also reported (82).

The endometrial wall thickness and cycle of sex hormones associations are well studied. Scientists observed an age-based relationship between altered endometrial wall thickness and BPA levels (83). Moreover, polycystic ovary syndrome (PCOS) patients exhibited higher BPA serum concentrations than healthy women and patients (84, 85). The researchers also detected BPA's potential role in PCOS and adverse pregnancy outcomes like premature delivery and miscarriage (86).

Males facing prolonged BPA exposure tend to have low sperm quality, sexual dysfunction, and impaired fertility. The amplitude of lateral head displacement (ALH), Wobble (WOB), Linearity (LIN), Mean Angular Displacement (MAD),

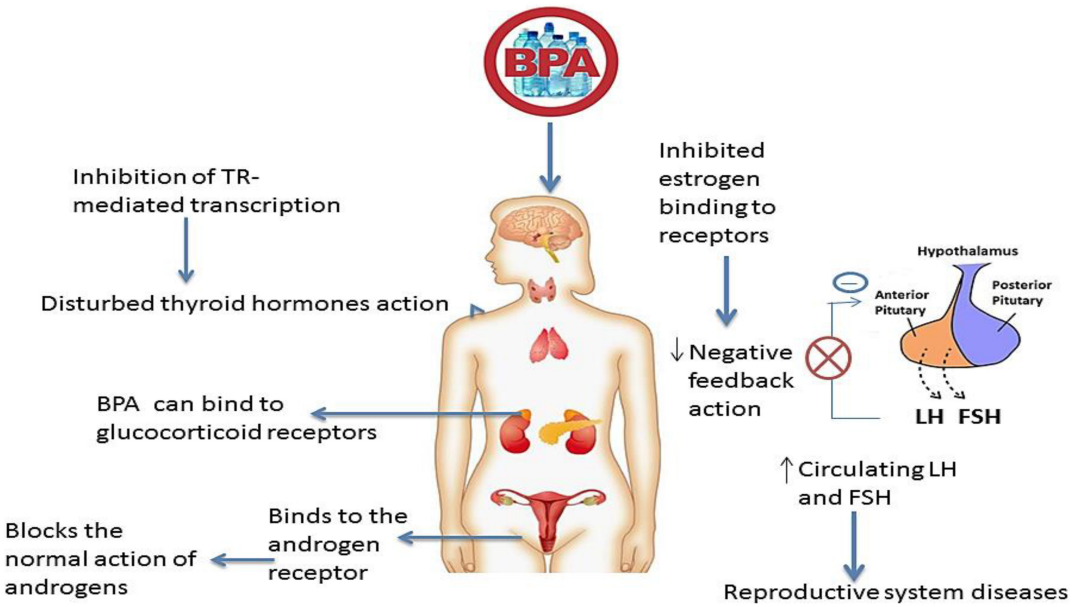

FIGURE 2
Bisphenol A as an endocrine disruptor. Estrogens negatively affect the release of follicle-stimulating hormone (FSH) and luteinizing hormones (LH). BPA can inhibit the estrogen binding to its receptors at the pituitary level, resulting in high levels of FSH and LH hormones in circulation. This can lead to reproductive system issues such as polycystic ovarian syndrome. BPA acts as anti-androgen by binding with androgen and glucocorticoid receptors and affecting their action. BPA can disturb the action of the thyroid hormone by inhibiting TR-mediated transcription of T_3-response genes.

sperm concentration, and association with BPA illustrated the fluctuated characteristics and velocity rate reduction. This array results in impaired reproductive function in males (87).

Carcinogenicity

The incidence of numerous cancer types is rising exceptionally and appears to be linked with BPA (**Table 4**). It includes breast (88), ovarian, uterus, prostate (89–91), and testicular cancer (92). The findings of the various *in vivo* studies on animals (i.e., mice, rats, etc.) concluded that the raised estrogenic activity depicts the carcinogenic mechanistic action of BPA (93). BPA's activation of tumorigenesis and cancerous cell development are still under experimentation (88). BPA stimulates cellular responses through binding to ER, although they reflect a weak affinity to each other (**Figure 4**).

TABLE 2 Effect of BPA on the endocrine system.

Specimen	Route of exposure	Findings/Health impact	References
Rats	Skin	BPA directly affects the central nervous system on exposure, primarily affecting CA3 pyramidal neurons and $GABA_A$ receptors.	(124)
Mice	BPA injection (125 mg/kg)	BPA exposure leads to endocrine disruption, which affects the immune system. BPA-induced steroid genesis and Nur77 gene expression in testicular Leydig cells.	(125)
Pregnant female mice	Oral	BPA affects the sex steroid hormone in the urogenital sinus of a fetus. Due to endocrine disruption, BPA increases estrogenic production and adversely affects the fetus's heart, kidneys, cerebellum, and ovaries.	(126)
Male offspring rats	Injection of BPA (low doses)	Hyperactivity and attention deficit due to endocrine disruption. In the basolateral amygdala, the BPA accumulation results in abnormal synaptic plasticity leading to these defects.	(127)
Rats offspring	Oral (during gestation and lactation)	Metabolic disruption due to raised glutamate and L-α-glutamyl- L-aspartic acid ratio in the hippocampus because the 2 metabolites are involved in the malate-aspartate metabolic shuttle. Myelination, growth, glial, and neuronal development alterations due to endocrine disruption.	(128)

The binding ability of the receptor to hold co-repressors is lost. As the regulation of co-regulators by the BPA–ER complex is disproportionate to the affinity of BPA to ER, the type and expression levels of ER-regulated targets are determinants for the tissue and cellular specificity of the BPA response. BPA can induce genomic responses at concentrations lower than the levels at which it is expected to bind to nuclear ERs (94).

TABLE 3 Impact of BPA on the reproductive system.

Specimen	Route of exposure	Findings	References
Female Sprague–Dawley rats	Oral	Significant hormonal disorders altered the structure and functions of the ovaries and uterus.	(129)
KGN ovarian granulosa-like tumor cell line	*In vitro*	Reduction in insulin-like growth factor 1 (IGF-1) induced by FSH and aromatase expression. BPA causes a reduction in granulosa cell DNA synthesis with no changes in DNA fragmentation, showing that BPA does not encourage apoptosis.	(130)
Pregnant women	Oral	Creatinine-identical BPA concentrations caused a reduction in reproducibility. BPA concentration was not altered by the intake of canned fruit, fresh vegetables, fruits, or fresh and frozen fish purchased from the store. High-molecular-weight phthalate and serum tobacco smoke metabolic compound levels were significantly linked with BPA levels.	(131)
Males	Oral	Increased serum total testosterone, prolactin, and estradiol resulted in a reduction in the androgen index.	(132, 133)
Males	Serum	Sexual desire and functionality were decreased in men, followed by premature ejaculation.	(134)
Males	Oral	A higher level of BPA in plasma and seminal plasma has a risk of an increased infertility level.	(135)
Males	Serum administration	The concentration of sperm was decreased, and sperm velocity ratios were increased, followed by a reduction in sperm motility and count.	(133, 136–138)
Females	Oral	The level of Luteinizing hormone and progesterone was increased; hence, the risk for PCOS also increased.	(139, 140)

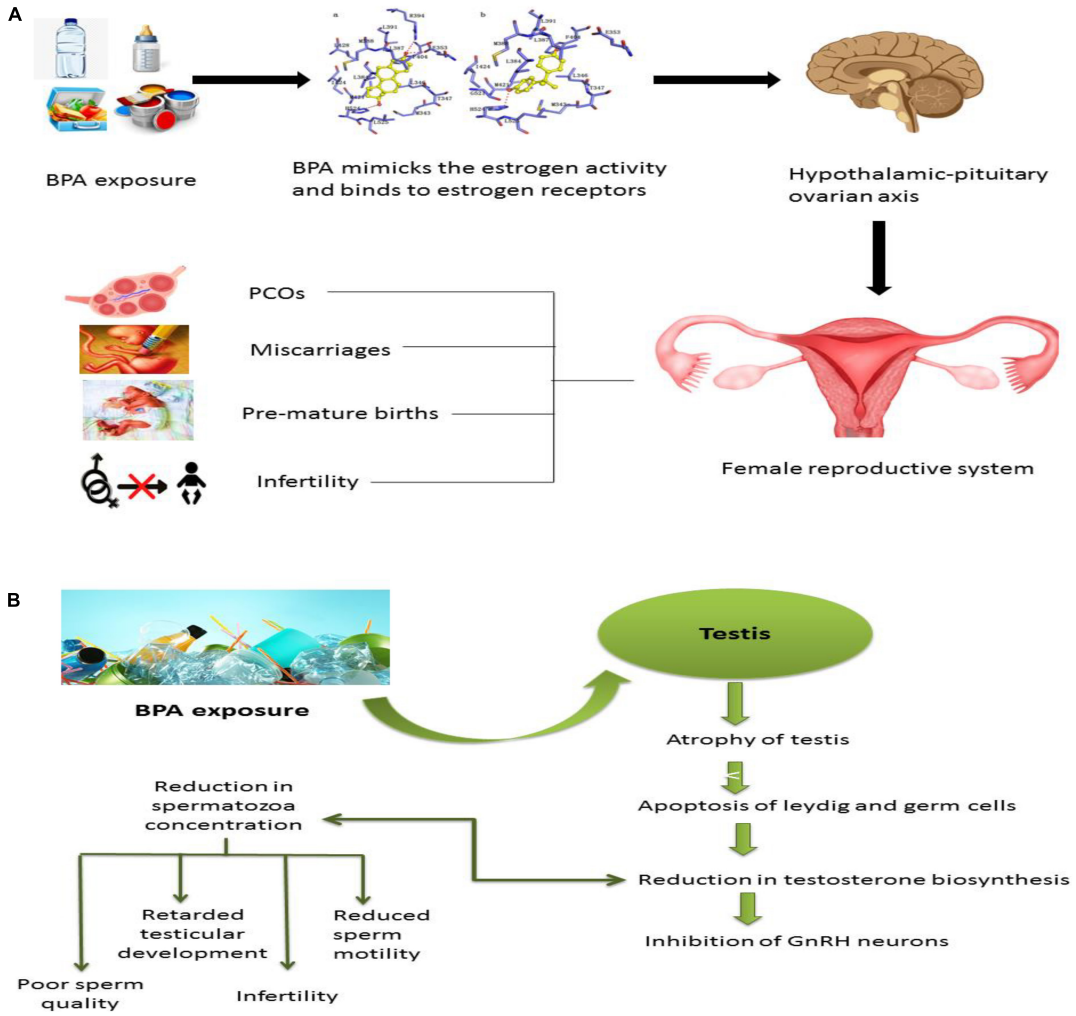

FIGURE 3
Effect of BPA on the reproductive system. **(A)** When exposed to BPA, females can develop fertility-related issues as it is very similar to estrogen structure and function. It binds to estrogen receptors and causes irreversible alteration to the hypothalamic-pituitary-ovarian axis. BPA will provoke estrogen and thus increase the chances of PCOs, delay puberty, miscarriages, endometriosis, premature births, and most of the time, BPA can cause infertility. **(B)** Exposure of males to bisphenol interferes with the reproductive system. BPA causes atrophy in the testis, apoptosis in Leydig cells and germ cells, and reduction in testosterone biosynthesis, which will either cause the reduction in spermatozoa reduction or inhibition of GnRH neurons. It causes sperm quality and quantity alterations, retardation of testicular development, infertility, and reduction in sperm motility.

Immunosuppressive action

Studies have revealed that oxidative stress, immune function, and inflammation are directly related to BPA exposure. The correlation between BPA and the induction of mitochondrial damage and cellular apoptosis resulted in systematic degradation (95–97), causing an alternation in immune cell populations and functioning of the innate and adaptive immune system owing to developmental BPA exposure (Figure 5).

Similarly, it also decreased T regulatory (Treg) cells and up-regulated pro-inflammatory and anti-inflammatory cytokines and chemokines. T1D development in females and males could be accelerated and decline on exposure to BPA (Table 5) (98).

Legislations

The primary source of contact for BPA is food for the general population. According to the United States Environmental Protection Agency (EPA), BPA's reference dose is 50 μg/kg BW/day. EFSA decreased this TDI dose from 50 μg/kg BW/day to 4 μg/kg BW/day in 2015 due to its harmful health effects (12).

A temporary TDI of BPA was set by the CEF panel that was 4 μg/kg BW per day. The board applied an uncertainty factor of 150 for this purpose for different body systems, including the reproductive system, metabolism, neurobehavioral, immune system, and mammary glands. It was done to assess the uncertainty along with inter and intraspecies differences. CEF

declared no harmful health impacts by comparing this t-TDI with the estimates of exposure from the diet for any age group and common health concerns from the combined exposure. Therefore, this estimation of exposure to non-dietary sources showed considerable uncertainty compared to dietary sources' estimates, imploring further research (99).

The rate of exposure from non-dietary sources, including thermal paper or medical devices based on current t-TDI derived by EFSA, was assessed by the SCENIHR or RAC, the Risk Assessment Committee. SCENIHR concluded that neonates in ICU, dialysis patients, and young children with prolonged medical treatment are at higher risk of getting adverse health effects from BPA as it may enter through systemic exposure after exposure to non-oral routes. But besides that, we cannot neglect the benefits of these devices (100). RAC also published and presented a restriction proposal for using BPA in the thermal paper under ECHA as an opinion of BPA's hazards on human health. The consumers were satisfied by RAC, ensuring the risk of BPA exposure through the thermal paper was controlled. Simultaneously, the chance of getting BPA exposure from cashiers was not declared adequately in control. Severe effects can be faced by pregnant female workers working in a high-exposure environment (101).

TABLE 4 Role of BPA in carcinogenicity.

Specimen	Route of exposure	Findings	References
Rat	Oral	The increased number of Leydig cells and proliferation was caused due to exposure to BPA during the Perinatal period.	(141)
Rat	Antenatal	Ductal carcinoma and ductal hyperplasias were developed due to increased BPA exposure during the perinatal period carcinoma *in situ* and malignant tumors.	(142)
Rat	Neonatal exposure	Polycystic ovary syndrome was reported due to the neonatal exposure to.	(143)
Mouse	Prenatal exposure	Cystadenomas, a high rate of progressive lesions of the oviduct and ovarian cysts, were observed due to Prenatal exposure to BPA.	(144)
Mouse	Neonatal exposure	Increased adenomyosis, cystic endometrial hyperplasia, and leiomyomas developed due to neonatal exposure to BPA.	(144)
Mouse	Oral	BPA exposure was studied in the renal xenograft model, and a high rate of adenocarcinoma of human progenitor cells and prostate intraepithelial neoplasia were reported.	(145)
Mouse	Perinatal exposure	Exposure to BPA during the perinatal period effect the offspring/infant greatly, and it leads to neoplastic lesions and hepatic pre-neoplastic.	(146)
Breeding C57Bl6 mice	Oral	Perinatal contact with BPA amplified the number of TEBs and the progesterone response of the mammary epithelial cells.	(147)
Non-human primates	Oral	BPA exposure to the fetus accelerated mammary epithelial development and a high rate of mammary buds' density.	(148)

For infants and children

Higher exposure rates of BPA are reported in Infants and children compared to adults. For breastfed infants, the average (95th percentile) BPA intake was 0.3 μg/kg body weight (BW) per day (1.3 μg/kg BW per day) for the age of 0 and 6 months. At the same time, 2.4 mg/kg BW per day (4.5 mg/kg BW per day) was reported for infants receiving formula from polycarbonate, according to WHO (47). European Union and Brazil have set the permissible limit of 600 μg/kg in infant foods. A study was conducted to assess the limit of BPA in infant formulas and reported the presence of BPA below the required level (0.2–10.2 μg/kg) (102). Another study estimated the BPA intake in different age groups and genders and concluded that their exposure was below the permissible limit (25 μg/kg of body weight/day) of Health Canada. However, dietary exposure to BPA for infants (0.22–0.33 μg/kg of body weight/day) was more than for adults (0.052–0.081 μg/kg of body weight/day). The increased intake was linked to the intake of canned and liquid milk-based infant formula (23).

Restrictions on the use of bisphenol A

Several restrictions on BPA use have been made after detecting its deteriorating health effects in different countries. The No Observed Adverse Effect Level (NOAEL) of BPA at a dose of 5,000 ng/kg body weight/day through the food intake was considered by the Food and Drug Administration in 2008 in the United States. Some EU Member States' also banned it in food packaging and containers for children up to 3 years of age, while some have prolonged this ban for other products. Denmark also prohibited using BPA in packaging materials, including cups, or bottles related to food, especially for a breast milk substitute, in 2010. The use of BPA in baby bottles was also banned after 1st March 2011 by EU Commission Directive No. 8/2011 as a preventive measure (103).

Likewise, baby pacifiers containing BPA were also banned in Austria in 2011. All the materials containing BPA that had

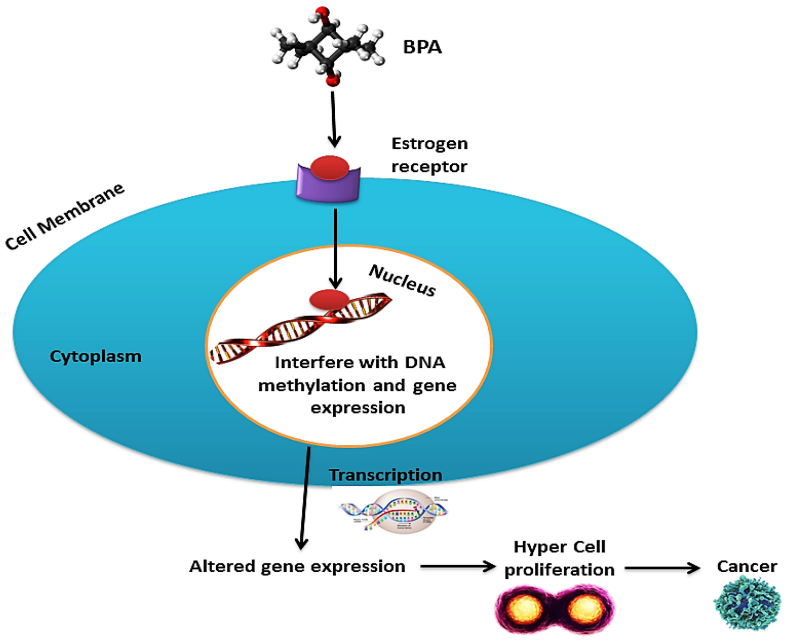

FIGURE 4
Carcinogenic activity of BPA; BPA interacts with the estrogen receptors and interferes with DNA methylation and gene expression after entering the nucleus. Thus altered gene expression leads to hypercell proliferation, which may lead to cancer.

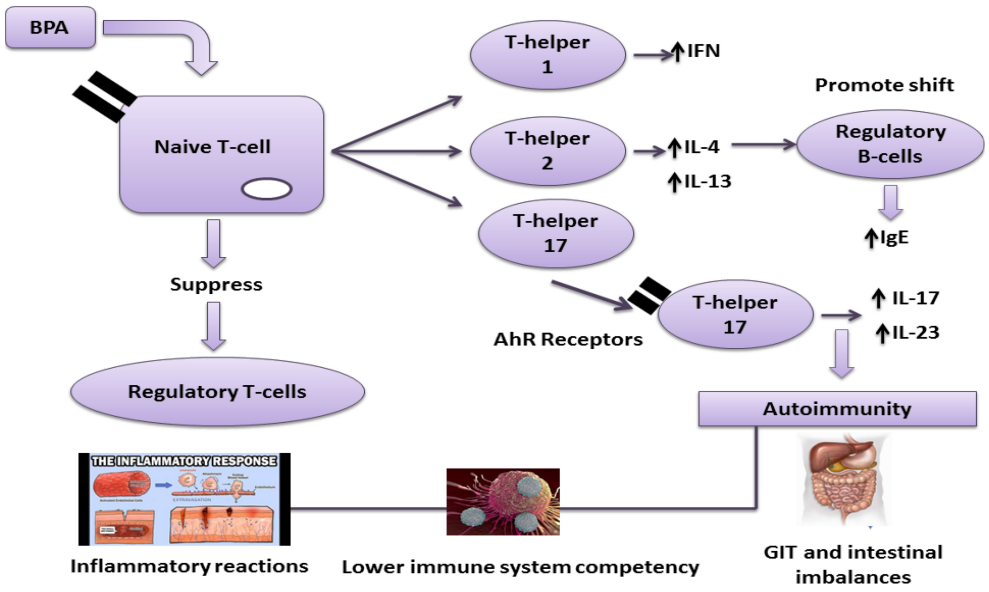

FIGURE 5
Effect of the BPA immune system BPA can promote autoimmunity *via* T-helpers 1, 2, and 17. Aryl hydrocarbon receptors (AhR) are involved in regulating immune responses, followed by the production of T-helper 17 a critical factor in T-cells in various autoimmune diseases.

a chance to contact food were suspended in France by passing a law in 2012 except for industrial equipment, such as tanks and pipes. They also introduced the labeling requirements for food items prepared for children and pregnant women. In 2013, Sweden banned the BPA-containing materials in lacquers of packaging linings for food prepared for children aged 0–3 years (104).

FDA restricted baby bottles and Sippy cups having BPA in 2012 and BPA derivatives in cans on infant formula in 2013 (41, 105, 106). The Commission also conducted an evaluation

TABLE 5 Effect of BPA on the immune system.

Specimen	Route of exposure	Findings	References
Pregnant women	Environmental (inhalation or dermal)	Concentrations of BPA in the mother's urine were reciprocally associated with odds of increased IL-33/TSLP.	(149)
Humans	Oral and environmental	BPA can harm the immune system's functions as assessed by CMV antibody levels and allergy or hay fever diagnosis.	(150)
Sprague–Dawley rats	Oral	Ten measurements out of 530 were different from vehicle controls and were primarily associated with dendritic or macrophage cell populations. BPA may have negatively affected the competency of the immune system.	(151)
Mice	Oral	BPA exposure *via* the oral route, in a given amount and for the shown contact period, has minor manipulation of features of the inflammatory response, stimulating immune-mediated diseases of the GIT.	(152)
Mice	Oral	Imbalances induced in intestinal and systemic immune *via* perinatal treatment, the appearance of inflammatory M1 macrophages in gonadal white adipose tissue with signs of aging, combined with a reduction in insulin sensitivity and an enhancement in weight gain.	(153)
Pregnant mice	Oral	Mother exposure to BPA modulated inborn immunity in mature offspring but did not damage the anti-viral adaptive immune response, which is dangerous for virus permission and endurance after influenza virus infection.	(154)
BALB/female mice	Oral	The chances of asthma increased when the mother was exposed to BPA. It might increase the airway hyperresponsiveness in their infants' lungs as they were bare to BPA before birth and after birth *via* breast milk compared to those exposed to BPA after birth or not treated with BPA at all.	(155)
Adult zebrafish	Oral	Down-regulation in the transcription of genes involved in enzymatic antioxidant defense and impaired anxiety and fear responses.	(156)
Larvae of Labeo rohita	Oral	Oxidative stress and suppressed NF-κB signaling pathway leading to immunosuppression.	(157)
Human granulosa KGN cells	*In vitro*	Damage to biomacromolecules-main targets of oxidative stress was significantly increased after treatment with BPA.	(158)
Adult rats	Oral	BPA-induced systemic oxidative stress change ROS-induced signaling pathways in the brain.	(159)

(started in December 2017) focusing on existing legislation on food packaging; this evaluation ended in 2019. To support the Commission's evaluation, the Joint Research Centre published a study (107) on the market condition of food packaging not coordinated in the EU. In December 2017, EFSA announced that a strategy was finalized to re-evaluate BPA toxicity with a working group for better results. BPA usage was restricted in thermal paper in January 2020, which would be through registration, evaluation, and authorization. Then restriction of chemicals regulation will decrease the use of BPA in all types of recycling packaging (108).

Alternatives to bisphenol A

There is a need to find alternative solutions to BPA due to the health hazards of this compound's use in packaging material. Several studies have been conducted in the previous few years to find the best suitable alternative to BPA having the same properties. Researchers have identified a few compounds that can be used as a replacement for BPA. The low estrogenic and endocrine potential of tetramethyl bisphenol F epoxy resin, bisguaiacol F, and tetramethyl bisphenol F diglycidyl ether is demonstrated in several studies. The researcher also suggested that further research is needed on this group of compounds to assess the potential possible effects of these compounds on human health.

Meanwhile, the authors suggested that they could be viable alternatives to BPA. These BPA substitute-based products are consumed under the label of "BPA-free." This term gives the impression that the products are safe, but the substitutes' safety is not fully verified (109, 110).

The increased restrictive rules for using BPA for human health and the environment have become a significant standard for substitution in research and industry (19, 111). Several "bisphenol analogs" have been produced to replace BPA in various applications (112). The most significant market shares are held by BPF (4, 4′-methylene diphenyl), BPS [bis (4-hydroxyphenyl) sulfone], and BPAF [2, 2-bis (4-hydroxyphenyl) hexafluoropropylene] (113–115). Reports and databases are available on the viability of these monomers as BPA substitutes. In Korea, BPS is used for thermal receipt papers and BPF as a water pipe coating agent instead of BPA (116). Although further research is needed to assess these compounds' potential effects, the authors suggest they could be viable alternatives to BPA (117).

Conclusion

Bisphenol A can cause multiple organ toxicity after entering the body through the respiratory, dermal, and gastrointestinal tract. It disturbs different cellular mechanisms and hormonal functions by binding with the receptors and activating downstream pathways. The outcomes of BPA exposure are cancers, endocrine disruptions, immunosuppression and reproductive defects. However, there are still ambiguities and many unanswered questions about BPA's metabolism and its toxic effects. There is a need to elaborate on the combined effects of BPA with other pollutants. Conclusively, BPA-free alternatives should be promoted to avoid these adverse consequences.

Future recommendations

It is obvious from the current review that more basic non-human primate research and clinical studies are needed to understand the too-complex mechanisms behind BPA activity fully. Nevertheless, several variations across species have been identified, although rats and mice have been demonstrated to be ideal models for investigating the causes of chronic human diseases. Additionally, additional research is needed as a preventative and precautionary measure, especially for developing fetuses and young children, as they are more vulnerable to the harmful effects of this prevalent compound in both developed and developing countries, which requires more attention even through public awareness campaigns. However, because humans are exposed to various pollutants, it is important to consider that BPA may have additive and synergistic effects with other widely used compounds.

Author contributions

MM, TT, AyS, and SM: conceptualization. AmS, MN, and FT: methodology. TT, FT, and SK: software. MM, TT, FT, and SM: writing—original draft preparation. MM, AyS, and X-AZ: writing—review and editing. X-AZ: supervision. SI: supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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Sea cucumber (*Acaudina leucoprocta*) peptides extended the lifespan and enhanced antioxidant capacity via DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1 in *Caenorhabditis elegans*

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The sea cucumber peptides (SCPs) from *Acaudina leucoprocta* were derived from the patented bio-enzyme digestion technology and the molecular weight of obtained SCPs was <10 kDa. In this study, we investigated the possible anti-aging effects of SCPs on the model of *Caenorhabditis elegans* and the underlying mechanisms. SCPs extend the average lifespan of nematodes by 31.46%. SCPs enhance the anti-stress capacity of *C. elegans* by improving heat resistance and mobility, Also, the accumulated potential oxidative stress inducers like lipofuscin and reactive oxygen species (ROS) were reduced to 40.84 and 71.43%. In addition, SCPs can increase the antioxidant capacity in nematodes by enhancing the activity of SOD and CAT and reducing MDA accumulation in nematodes to 32.44%. Mechanistically, SCPs could mediate DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1 axis to improve antioxidant capacity and extend lifespan in nematodes. Taken together, these findings provide a direction for the anti-aging effects of sea cucumber peptides and new insights into the further purifications of SCPs and future research on aging.

KEYWORDS

sea cucumber peptide, *Caenorhabditis elegans*, anti-aging, antioxidant, insulin/IGF-1 signaling

Introduction

Aging is typically characterized as the slash of the body's physical and psychological adaptability to outside stress, which is induced by the combined actions of holistic physiologic factors like stem cell decline, DNA degradation, and external dietary and mental factors (1, 2). During the aging process, the functions of multiple organisms will decline, which could induce aging-related diseases such as hypertension, hyperlipidemia, diabetes, neurodegenerative diseases, cardiovascular diseases, and even worse, cancer (3). Therefore, healthy life extension effectively delays aging and reduces the prevalence of aging-related diseases (4).

In ancient times, sea cucumber was a precious food for nutrition supplementation and physical fitness elevation. According to *Compendium of Materia Medica*, sea cucumber is endowed with warm and nourishing properties, and its effects are almost the same as that of ginseng whose nourishing effect on the kidney meridian, has been well-documented, benefits the essence, dispel phlegm and saliva, diuretic, invigorate blood and impotence, and cure the spread of ulcers (5). *Acaudina leucoprocta* is a kind of edible sea cucumber distributed in Zhejiang, Guangdong, Fujian Province, and northwest Australia, which has become a new species of economic interest in China for its untapped nutrient potential and its abundance (6). *Acaudina leucoprocta* is abundant and low-cost, with proven technology for heavy metal removal (7), providing a low-cost alternative to the current expensive peptide resources. Sea cucumber peptides are frequently useful for preparing soup and drinking in our life (8). Sea cucumber peptides (SCPs) obtained from *A. leucoprocta* by protease hydrolysis, and isolated and purified by neutral proteinase complex are small molecule peptides (9). The study of the bioavailability of bioactive peptides after *in vitro* digestion and first metabolism revealed that all peptides were transported across the intestinal cell layer to varying degrees, and are not metabolized by the liver (10). It has also been shown that the diversity of peptides increases after *in vitro* gastric and small intestinal digestion (11). Therefore, the peptides are stable, not metabolized, and not degraded during human digestion, increasing diversity. It has been reported that these kinds of SCPs are endowed with anti-oxidation, anti-aging, anti-fatigue, and immunomodulatory properties (12). Moreover, sea cucumber peptides with a high degree of hydrolysis obtained by biological enzymatic hydrolysis have excellent solubility, stability, emulsification, easy digestion, and absorption characteristics good nourishing product (13).

C. elegans, a classical model organism, has a clear genetic background, a body structure accessible for detection, and short life history. It applies to utilizing molecular biology methods for genetic intervention and behavioral tracing (14). In *C. elegans*, IIS plays a key role in the regulation of development, metabolism, and aging (15). The IIS is a key pathway in the delayed aging of SCPs with the following key genes, (*daf-16*, *daf-2*, *sod-3*). SODs acted as a superoxide anion radical remover, which could respond to oxidative stress and affect the lifespan of nematodes (16). In addition, *daf-2* is a key gene for ROS resistance and increased lifespan (17). Research shows that *daf-16* is a downstream gene of *daf-2* and an upstream gene of *sod-3* (18, 19). The *old-1* and *pept-1* are closely linked to insulin pathway-related genes. OLD-1 signaling was specific for longevity and stress resistance and may transduce signals to DAF-16 (20, 21). PEPT-1 is an electrogenic symporter that couples substrate transport to proton movement across the membrane, therefore leading to an acidification of the cytosol (22). Genetic studies indicated that *pept-1* interacts with both the *daf-2*/insulin- and *let-363*/TOR-signaling pathways to regulate lifespan and with the *daf-2* pathway to influence stress response (23). Therefore, nematodes were used to study the anti-aging activity of SCPs and their mechanism. However, the possible effects of SCPs on DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1 remain unelucidated.

We examined the effect of SCPs on the lifespan and stress resistance of *C. elegans*. The discovery of the anti-aging effect and the mechanism of sea cucumber peptides (*Acaudina leucoprocta*), proposed a new direction for future research on peptide-related anti-aging mechanisms: DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1, and also provided the basis for the purification of sea cucumber peptides in the future.

Materials and methods

Strains and reagents

Sea cucumber peptides were provided by Hangzhou Kang Yuan Food Technology Co Ltd (Hangzhou, CHN), Metformin (Met), β-nicotinamide mononucleotide (NMN), Methyl viologen (paraquat), and 5-fluoro-2'-deoxyuridine (FUDR) and were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, CHN). Total superoxide dismutase (SOD) assay kit, catalase (CAT) assay kit and malondialdehyde (MDA) assay kit were provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, CHN). Reactive oxygen species (ROS) was obtained from Vigorous Biotechnology Beijing Co., Ltd. (Beijing, CHN).

C. elegans (wild-type N2), and mutant strains CF1038 [*daf-16(mu86)I*], TJ356 [zIs356 IV (*daf-16p::daf-16a/b::GFP* + *rol-6(su1006)*)], CB1370 [*daf-2(e1370)III*], CF1553 [(pAD76) *sod-3p::GFP*], SN5[*old-1(mk1)II*], BR2742[*pept-1(lg601)X.*], RB1159[*tyr-3(ok1194)I.*], RB1985[*acox-1.5(ok2619) III.*].were purchased from the Caenorhabditis Genetics Center (CGC). The expression of Green Fluorescent Protein (GFP) and the lifespan of the mutant nematodes were recorded. Escherichia coli OP50 (E. coli OP50) was obtained from SunyBiotech.

Determination of amino acid composition

Gel exclusion chromatography in HPLC was used to TSK-GEL G2500PWXL as the separation column to analyze the molecular weight distribution of SCPs. The amino acid composition of SCPs was measured by HPLC (Thermo Scientific, Waltham, USA). Briefly, 20 mg of samples were dissolved in 6 M HCl (1 mL), followed by hydrolysis at 150°C for 1.5 h. After the completion of hydrolysis, derivation. After filtration with a 0.22 μm membrane filter, the sample was analyzed by HPLC on the AQ-C18 column (4.6 × 250 mm, Welch Co., Ltd, Shanghai, China) at 254 nm using the mobile phase of A (acetic acid/sodium acetate buffer solution) and B (80% acetonitrile) at 1.0 mL/min.

Cultivation of *C. elegans*

The L4 stage of nematodes was transferred to NGM plates containing SCPs-L (0.0625 mg/mL), SCPs-M (0.125 mg/mL), SCPs-H (0.25 mg/mL), Metformin (Met, 40 μmol/L), β-nicotinamide mononucleotide (NMN, 0.25 mg/mL) and FUDR (1 mg/mL) to prevent spawning. For the synchronization of *C. elegans*, put the pregnant adults in the NGM medium coated with OP50 and observe their oviposition status. The pregnant adults are picked out after oviposition, and the eggs are cultured for 48 h to L4 stage adults at 20°C to get the same-aged worms for further experiments (16).

Lifespan assay

All lifespan assays were carried out at 20°C. The transfer day was designated as day 0. Worms were transferred another day to fresh extracts or control plates until day 7, which was thought to be the adulthood of the *C. elegans*. During the experiment, worm bags and abnormal dead worms were removed. Triplicate plates were used for each group (30 per plate). During the lifespan test, the movements of the *C. elegans* were recorded at 18, 20, 22, and 24 days.

Stress test

Same-aged *C. elegans* were picked onto the medicated plates and cultivated for 7 days. For the thermotolerance assay, nematodes were placed in 37°C conditions for 13 h and then the number of dead worms was counted. Triplicate plates were used for each group (30 per plate). To test the resistance of *C. elegans* to paraquat, nematodes were exposed to 140 mM paraquat. The number of surviving worms was counted every 2 h until all the worms died.

Lipofuscin determination

To determine the lipofuscin level, on 13 days of treatment, worms treated with SCPs were placed on 1% agarose pads on glass slides and anesthetized with Levamisole Phosphate (6.75 μM). The worms were visualized under a fluorescence microscope (Axio Scope. A1, American), and were measured using Image J 1.8.0 software.

ROS detection

After day 7 of treatment with SCPs, the nematodes were washed with nutrient buffers (M9), and then 100 μL of the prepared ROS fluorescent agent was added for fluorescent excitation at 20°C for 5 h. The worms were visualized under a fluorescence microscope (Axio Scope. A1, American). The fluorescence intensity of the worms was measured using Image J 1.8.0 software (24).

Antioxidant detection in *C. elegans*

The nematodes treated by SCPs were lysed with double distilled water in an ice bath after 7 days. The activities of SOD, MDA, and CAT content were determined (25).

Real-time quantitative PCR

Total RNAs of nematodes administration (7 days) were isolated using an RNA extraction kit (Aidlab Biotechnologies Co. Ltd, Beijing, China) and cDNAs were synthesized using a reverse transcription kit (Takara, Biotechnology Co. Ltd, Dalian, China). The qPCR reaction was performed on a qTOWER 2.0 PCR system (Light Cycler, Switzerland) using SYBR Green PCR Master Mix (Takara). Relative expression levels of genes were calculated using the $2^{-\Delta\Delta CT}$ method, and the gene *act-1* was set as the internal reference, shown in Table 1. The best primers for genes of *C. elegans* were obtained from the qPrimerDB-qPCR Primer Database (26).

Statistical analysis

Data are reported as means ± standard deviations (SD) or standard errors (SEM). Assays for each experiment were performed independently at least three times. At least three separate assays were run for each experiment. The Kaplan-Meier test was used to plot survival curves using GraphPad Prism 8.0 (GraphPad Software, Inc., CA, USA), and SPSS 21.0 was used to do the log-rank test. One-way analysis of variance (ANOVA) analyses

TABLE 1 Primer sequences.

Gene	Gene ID	Direction	Primer sequences ($5'$-$3'$)
Caenorhabditis elegans act-1	1,79,535	Forward	GCAAGAATACGACGAGTCCG
		Reverse	TAGAAAGCTGGTGGTGACGA
Caenorhabditis elegans daf-2	1,75,410	Forward	CCCAAGTTTGAGCTCCAAGAG
		Reverse	TCGTCATCGTTCTGTCTGCAT
Caenorhabditis elegans daf-16	1,72,981	Forward	GCTGCTGCCTTCACTCTCAT
		Reverse	GATGACTGGCCACTGGTGTG
Caenorhabditis elegans sod-3	1,81,748	Forward	TCTCCAACCAGCGCTGAAAT
		Reverse	CCAGAGCCTTGAACCGCAAT
Caenorhabditis elegans old-1	1,71,737	Forward	CACCAGAAAGCTCCGTTCAGA
		Reverse	ACTGAGGAAGAGGAATCAAGTG
Caenorhabditis elegans pept-1	1,80,919	Forward	AAACTTTGCCATGTGCCGTC
		Reverse	ACAGCCGGTTGGGAACTAAG
Caenorhabditis elegans tyr-3	1,72,472	Forward	TCATGCGCCCATTTACACCA
		Reverse	CGAGGAGCTCCGTGTGATTT
Caenorhabditis elegans acox-1.5	1,76,353	Forward	AACTGAGTGGTGGCTGATGG
		Reverse	GATTGGTTCCGTGTCCGAGT

This table includes all primers used in RT-qPCR experiments.

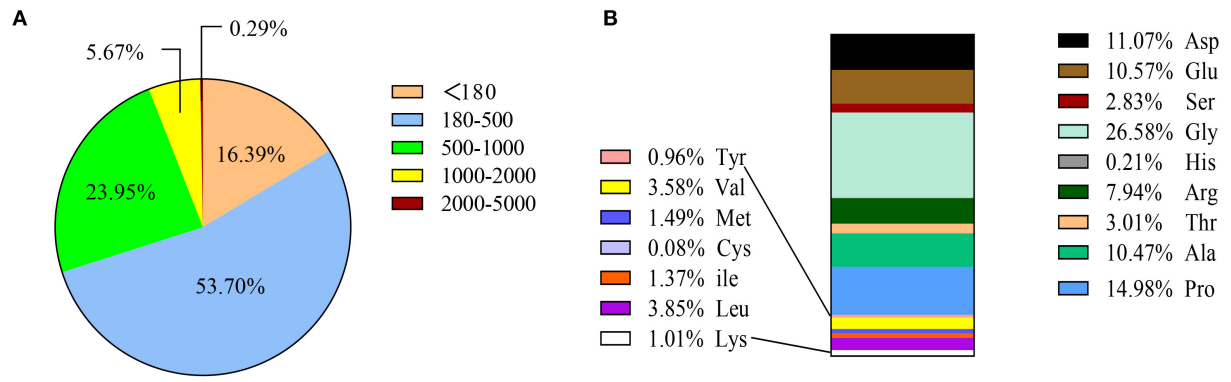

FIGURE 1
Amino acid composition and MW distribution of SCPs. **(A)** MW distribution of SCPs. **(B)** Amino acid compositions of SCPs.

with the Tukey-Kramer test or *t*-test was carried out to detect the statistical significance with a significant level of $\alpha = 0.05$ (27).

Results

Amino acids composition and Molecular weight distribution of SCPs

The molecular weight of SCPs <1,000 Da accounted for 94.04% of the total, of which molecular weights <180 Da, 180–500 Da, and 500-1000 Da respectively accounted for 16.39, 53.70, and 23.95%, and molecular weight >2,000 Da only accounted for 0.29%. This shows that sea cucumber peptides are small molecule peptides that are helpful for human digestion and absorption. SCPs are also high in glycine (Gly), proline (Pro), aspartic (Asp), and Alanine (Ala) (Figure 1).

SCPs extended the lifespan of *C. elegans*

The results showed that the survival rate of the nematodes pretreated with SCPs was much higher than the control group (Figure 2). As shown in Table 2, the mean lifespan of SCPs-L, SCPs-M, and SCPs-H groups was increased by 6.50, 31.46, and 21.30%, compared to the control group.

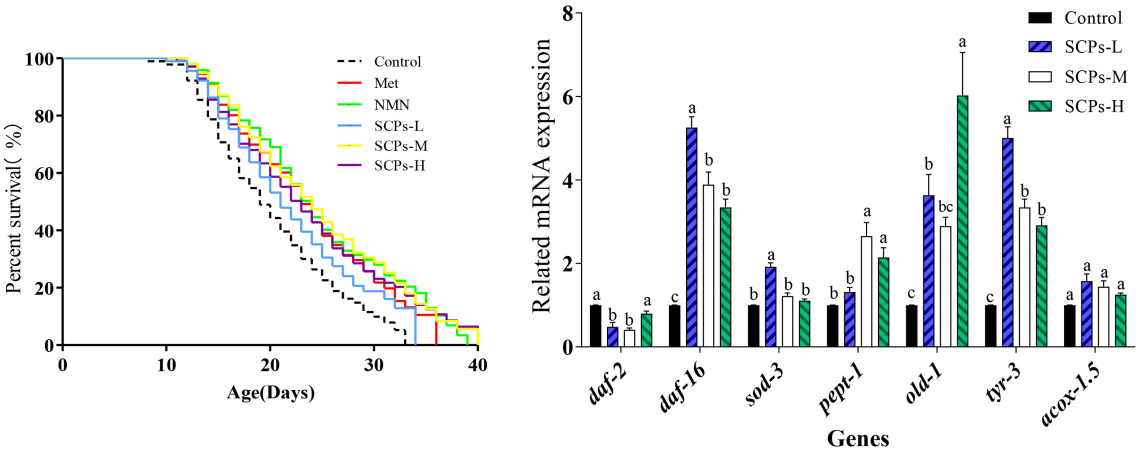

FIGURE 2
Effects of SCPs on the lifespan of wild-type *C. elegans* under normal conditions. Statistical analyses were carried out using GraphPad software. A Kaplan-Meier lifespan analysis was carried out, and *p-values* were calculated using the log-rank test. In all statistical analyses, $p < 0.05$ was accepted as statistically significant.

TABLE 2 Effects of SCPs on the life span of wild-type *C. elegans* under normal conditions.

Strain (solvent)	Maximum lifespan (d)	Life extension rate (%)	Mean lifespan (d)	Life extension rate (%)
N2 (Control)	28.75 ± 1.89^{b}	0	17.81 ± 1.39^{b}	0
N2 (Met)	31.75 ± 2.63^{ab}	10.43	19.65 ± 3.02^{ab}	10.37
N2 (NMN)	35.00 ± 5.89^{ab}	21.74	22.90 ± 1.46^{a}	28.60
N2 (SCPs-L)	29.75 ± 3.40^{ab}	3.48	19.56 ± 2.35^{ab}	6.50
N2 (SCPs-M)	38.00 ± 4.20^{a}	32.17	23.41 ± 1.91^{a}	31.46
N2 (SCPs-H)	34.50 ± 4.35^{ab}	20.00	21.60 ± 2.60^{ab}	21.30

Different letters correspond to statistically significant differences ($p < 0.05$) between groups in the same column.

Meanwhile, the maximum lifespan of the three groups was increased by 3.48, 32.17, and 20.00%, compared to the control group. More importantly, after administering SCPs-M, the mean and maximum lifespans were prolonged by 21.09 and 2.86%, 21.74, and 10.43%, compared to the Met and NMN groups.

SCPs enhanced the stress tolerance and motion ability of *C. elegans*

At 10 h, there was a significant difference between the levels of SCPs-M and positive controls were consistent (Figure 3A). The survival rates of SCPs groups were considerably greater than the control group during paraquat stress and heat stress (Figures 3B,C). At the same time, we detected whether the increased lifespan was accompanied by an improvement in the vitality of the nematodes. We tested the motility of nematodes treated with SCPs for 18, 20, 22, and 24 days, as shown in Figure 3D. The mortality of the SCPs treatment group was lower than the control group, which suggested that SCPs did not affect muscle contraction and other functions of N2. The majority of worms could move on their own by day 18. By day 20, only 40.81% of the worms in the control group possessed class A motility, compared to 42.86, 60.00, and 55.41% in the SCPs-L, M, and H treatment groups, respectively. On days 22 and 24, 24.07 and 30.99% of worms had class A and B motility in the SCPs-M treatment group more than those in the control group. These findings suggested that SCPs could improve *C. elegans* capacity for mobility and stress tolerance.

SCPs increased the antioxidant effect and decreased lipofuscin accumulation of *C. elegans*

The autofluorescence of lipofuscin can be used to estimate the aging of nematodes. As shown in Figures 4A,B, SCPs, Met, and NMN can effectively reduce lipofuscin in nematodes. The lipofuscin content in N2 worms treated with SCPs-L, M, and H was lower by 35.48, 40.84, and 40.41%, respectively, compared to the control group. Increased ROS levels may

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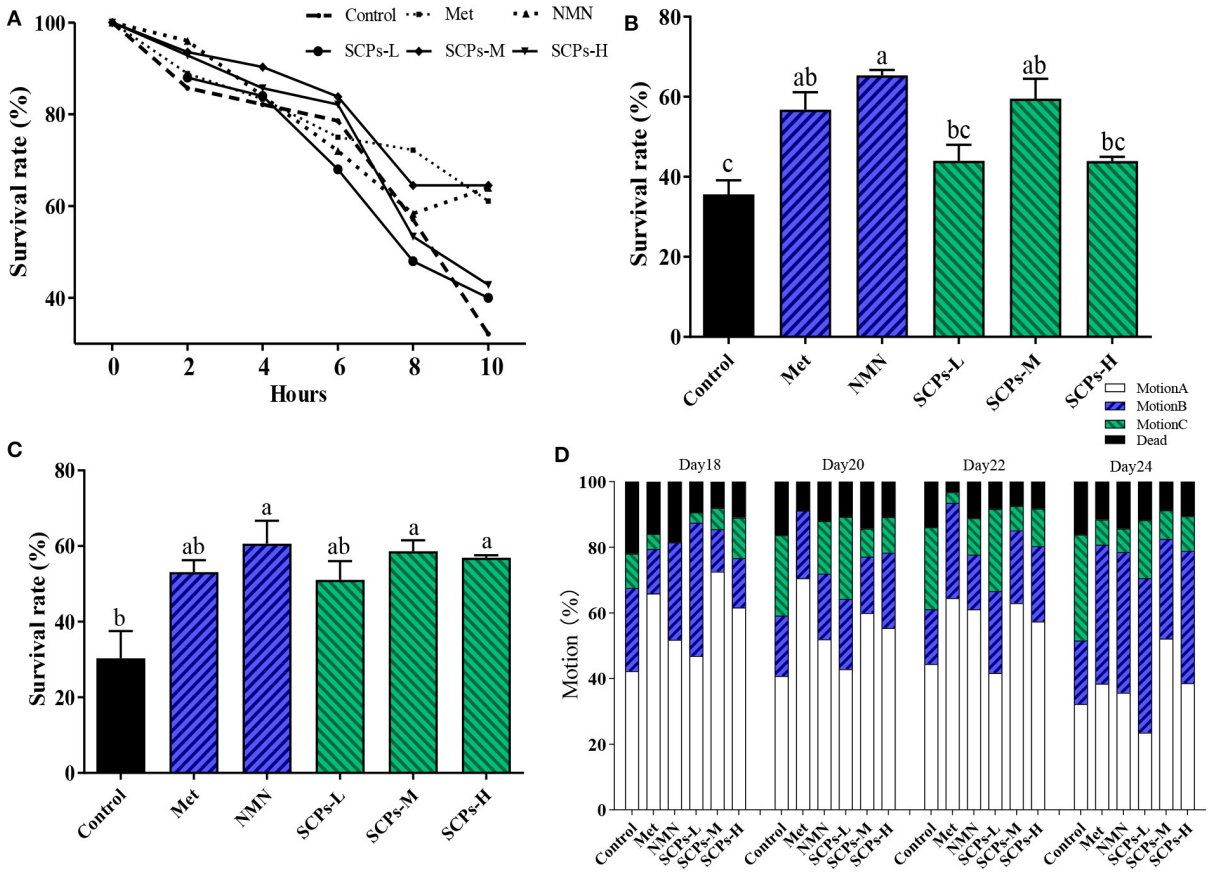

FIGURE 3
Effects of SCPs on the stress and motion ability in N2 over time. **(A)** Effect of SCPs on N2 survival over time under paraquat-stimulated. **(B)** Effect of SCPs on N2 survival under paraquat-stimulated. **(C)** Effect of SCPs on the survival of N2 at 37 °C thermal shocks. **(D)** The effect of SCPs on the mobility of N2. The movement is the free crawling as action A; the body twisting or head twisting after the platinum wire is lightly touched as action C; between the two is action B. Different letters correspond to statistically significant differences ($p < 0.05$) between groups.

damage cell structure significantly, so prevention of excessive ROS accumulation has been shown to be an efficient strategy to delay aging. When the nematodes were pretreated with SCPs, the fluorescence of N2 was substantially reduced as compared to the control group. Among these, the SCPs-M group had the lowest ROS content at the same level as the NMN group (Figures 4C,D). The activities of SOD, MDA, and the CAT contents are important indexes of defense against potential oxidative damage in organisms. As shown in Figure 3E, SOD activities were significantly increased by SCPs treatment (by 1.4–1.5-fold in the SCPs-treated groups compared with the control group). The MDA contents were 10.18%-32.44% lower in the SCPs treated groups than in the control group. The CAT activities were significantly increased by SCPs treatment (by 1.6–2.1-fold in the SCP-treated groups compared with the control). These results indicated that SCPs reduced oxidative deposition in the body and enhanced the antioxidant capacity of nematodes.

SCPs up-regulated *daf-16*, *sod-3*, *pept-1*, *old-1*, *tyr-3*, *acox-1.5*; down-regulated *daf-2*

To explore the mechanisms of these beneficial effects, we analyzed the expression levels of genes related to longevity, stress resistance, apoptosis, or the impact Dauer period (Figure 5). The expression level of genes in the control group was set to 1. In SCP s-treated groups, the relative expression levels of *daf-2* ranged from 0.41 ± 0.08 to 0.79 ± 0.11, *daf-16* and *sod-3* were SCPs-dose-dependent; the relative expression levels of *pept-1* ranged from 1.31 ± 0.20 to 2.65 ± 0.57. Those of *old-1* ranged from 2.89 ± 0.37 to 6.02 ± 1.78. Other genes showing increased expression levels after SCPs treatment included *tyr-3*, and *acox-1.5*. It is hypothesized that *pept-1* may regulate *daf-16* and its down-regulated genes through *daf-2* (28).

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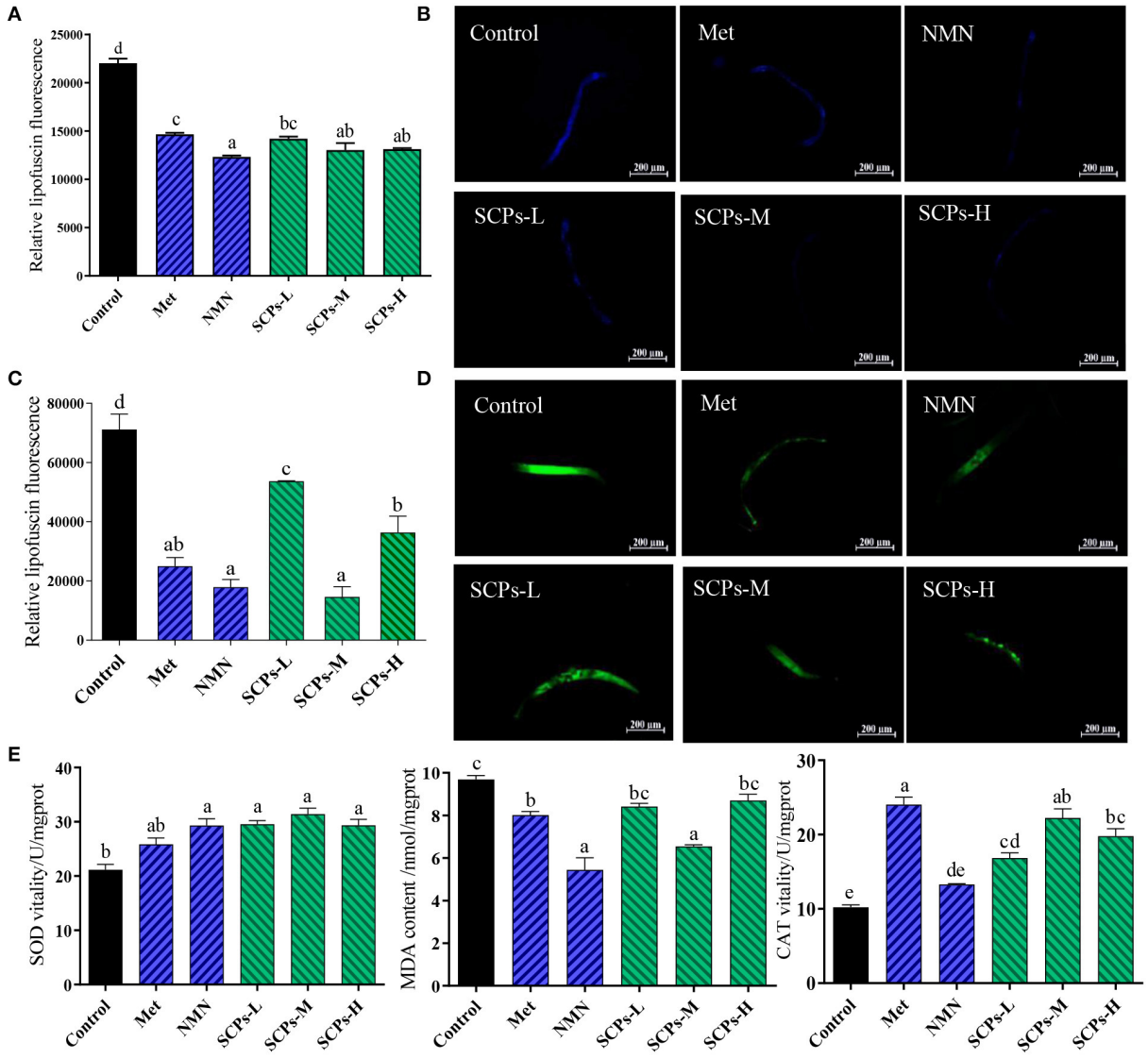

FIGURE 4
Effect of SCPs on lipofuscin, ROS accumulation, and internal oxidation in N2. **(A)** The bar shows a gray value on lipofuscin in N2 after treatment with SCPs. **(B)** The typical fluorescent pictures of lipofuscin. **(C)** The bar shows a gray value on ROS in N2 after treatment with SCPs. **(D)** The typical fluorescent pictures of ROS accumulation. **(E)** The effects of SCPs on SOD, MDA, and CAT of N2 under normal conditions. Different letters correspond to statistically significant differences ($p < 0.05$) between groups.

daf-16, *daf-2*, *sod-3*, *pept-1*, and *old-1* genes were required for the benefits of SCPs in *C. elegans*; *tyr-3* and *acox-1.5* genes were not required for the benefits of SCPs in *C. elegans*

The GFP signal was shown as cytosolic, intermediate, and nuclear localization in Figure 6A. The results illustrated a significant rate of higher nuclear location up to 44% and lower cytosolic location down to 57% compared to the control (Figure 6B). Furthermore, SCPs had no significant effect on lifespan in *daf-16* null mutants, control (11.28 ± 0.34), SCPs-L (11.67 ± 0.35), SCPs-M (12.22 ± 0.06), SCPs-H (12.19 ± 0.21). These data suggested that the deletion of *daf-16* could affect the role of SCPs (Figure 6C). These results conveyed that SCPs might promote DAF-16 translocation to enhance the effects on stress resistance and longevity.

The inhibited *daf-2* or activated *daf-16* could be the key target for regulating IIS to delay aging in nematodes. SCPs could effectively reduce the accumulation of lipofuscin

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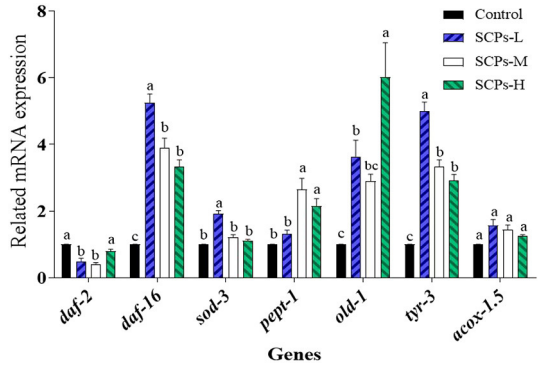

FIGURE 5
Effect of SCPs on the relative expression levels of anti-aging related genes in *C. elegans*. Data were expressed as the mean ± SD. Different letters correspond to statistically significant differences ($p < 0.05$) between groups in the same gene.

(Figures 6D,E) and extended lifespan in *daf-2* null mutants with no significant difference, control (22.78 ± 0.88), SCPs-L (20.72 ± 0.91), SCPs-M (22.80 ± 0.30), SCPs-H (22.11 ± 0.91), which suggested that *daf-2* played an important role in delaying aging in SCPs (Figure 6F). SOD-3 can remove excess superoxide and free radicals in the body (29). In comparison to the control group, SCPs-L, M, and H treatments increased the expression of SOD-3: GFP by 76.21, 90.98, and 26.89%, respectively (Figures 6G,H). SCPs had no significant effect on lifespan in *pept-1* null mutants, control (21.35 ± 0.83), SCPs-L (20.43 ± 0.88), SCPs-M (20.77 ± 0.26), SCPs-H (21.08 ± 0.16) (Figure 6I). OLD-1 was transcriptionally regulated by the DAF-16 forkhead transcription factor, and both were expressed in the whole body. SCPs were able to significantly reduce the lifespan of *old-1* null mutants compared with the control group, control $(14.62 \pm 0.55)^{a}$, SCPs-L $(13.02 \pm 0.23)^{b}$, SCPs-M $(11.32 \pm 0.12)^{c}$, SCPs-H $(12.39 \pm 0.25)^{bc}$ (Figure 6J). These results suggest that SCPs extend the *C. elegans* lifespan and improve antioxidant ability via *daf-16*, *daf-2*, *sod-3*, *pept-1*, and *old-1*.

SCPs up-regulated *tyr-3* and *acox-1.5* by other ways of anti-aging, which suggests SCPs might mediate the reduction of apoptosis and regulation of the Dauer phase in nematodes, by validating their corresponding mutants, we found that SCPs extended, respectively the lifespan of *tyr-3* null mutants 19.91, 14.72, 10.89% than the control group (Figure 6K). And also, SCPs extended the lifespan of *acox-1.5* null mutants, control $(14.07 \pm 0.21)^{b}$, SCPs-L $(16.14 \pm 0.20)^{a}$, SCPs-M $(16.65 \pm 0.11)^{a}$, SCPs-H $(16.21 \pm 0.13)^{a}$ (Figure 6L). This means SCPs are not able to act by inhibiting apoptosis and regulating the Dauer phase.

In summary, the following diagram of the mechanism of SCPs regulating aging was obtained in Figure 7.

Discussion

The population is aging, which is a severe issue in recent decades. Anti-aging product research is exploding, and biologically active peptides to slow down the aging process has become a hot topic. Bioactive peptides are effective and have been well studied, such as the rice bran peptide KF-8 improves the health span of *Caenorhabditis elegans* (30) and the walnut protein exhibited an excellent anti-photoaging effect (31). As examples of animal peptide sources, chicken bone collagen peptides have been shown to dramatically reduce the signs of skin aging (32), and the crimson snapper scales peptides effectively extended the lifespan and improved the motor ability of both male and female Drosophila (33). In the sea cucumber peptides research, SCPs (*Stichopus variegates*-derived peptides) significantly reduced D-gal-induced oxidative damage in mice by triggering SOD and GSH-Px and obstructing lipid peroxidation and protein oxidation (34, 35). Additionally, SCPs (*Apostichopus japonicas*-derived peptides) could also alleviate oxidative stress in neuroblastoma cells and improve survival exposed to neurotoxic paraquat in *C. elegans* (36). As a result, in the wide-type N2 and mutant model, we found that SCPs (*Acaudina leucoprocta*-derived peptides) have positive effects on health promotion, lifetime extension, and the likely underlying mechanism. As such, we detected the effects of SCPs on health promotion, lifespan extension, and the probable underlying mechanism in the wide-type N2 and mutant model. SCPs extend the lifespans of *C. elegans*, the mean lifespan of SCPs-M, and H were all higher than those of the positive Met, and the SCPs-M was higher than the NMN. The average lifespan of nematodes can be extended by up to 31.64% more than the 20% extension of sea cucumber *Apostichopus japonicus* (6). The longevity elevation of *C. elegans* is always accompanied by an improvement in stress resistance capacity (37). As anticipated, SCPs can significantly increase nematodes' resistance to heat and oxidative stimulation and decrease the build-up of lipofuscin and ROS in *C. elegans*. SCPs, like the sea cucumber *Apostichopus japonicus*, could boost SOD and CAT activities and decrease MDA accumulation in terms of anti-oxidation *in vivo*.

The IIS is characterized by diminishing insulin signaling, enhancing insulin sensitivity, and reducing plasma insulin-like growth factor-1 levels. This is a process involved in the formation of Dauer larvae (38). A DAF-16 transcription factor is crucial in regulating lifespan and stress resistance in nematodes (39). Under normal physiological activity, *daf-16* activity is at low levels, however, the *daf-2* mutation may restore *daf-16* activity, allowing adults to live more than twice as long as N2 (40). Therefore, we further studied the role of the insulin signaling pathway in regulating aging, and the results showed that treatment with SCPs improved the extension of the lifespan of *daf-2(e1370)* mutants, promoted the transfer of DAF-16 into the nucleus, up-regulated *pept-1* and increased the expression of the *daf-16* downstream gene, including *sod-3*, *old-1*. We have

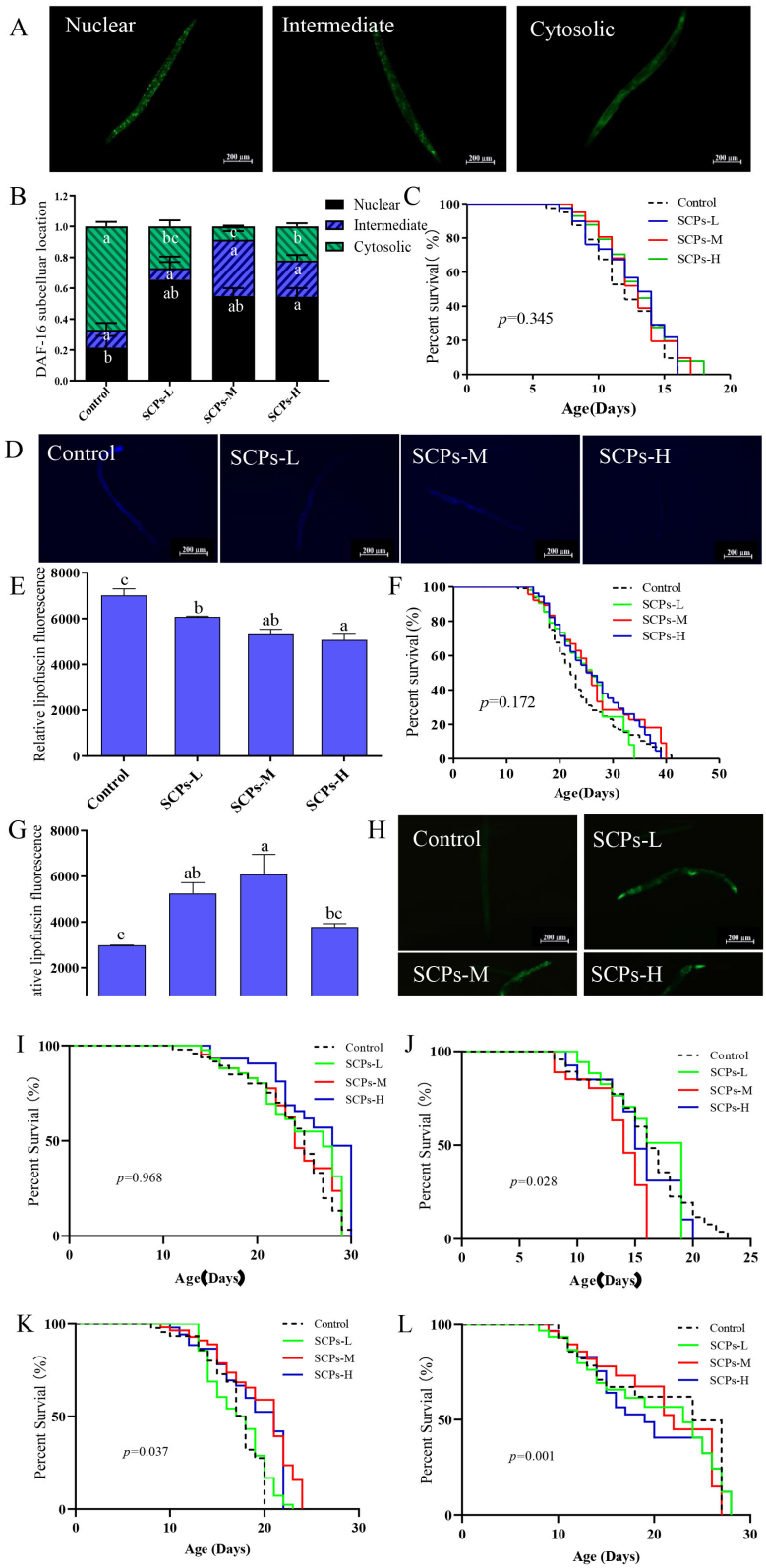

FIGURE 6
SCPs extended the lifespan of *C. elegans* through the *daf-2*,*daf-16*,*sod-3*,*old-1*,*pept-1*. SCPs extended the lifespan of *C. elegans* and could not through the *tyr-3* and *acox-1.5*. **(A)** Distribution of DAF-16 "cytosolic," "intermediate" and "nuclear," **(B)** The proportion of DAF-16: GFP

(Continued)

FIGURE 6 (Continued)
"cytosolic," "intermediate" and "nuclear," **(C)** Effect of SCPs on the lifespan of *daf-16* mutants. **(D)** The lipofuscin accumulation was presented in fluorescent pictures. **(E)** The bar shows a gray value on lipofuscin in *daf-2(e1370)* after treatment with SCPs. **(F)** Effect of SCPs on the lifespan of *daf-2(e1370)* mutants. **(G)** The bar shows a gray value on fluorescent in (pAD76) *sod-3p: GFP* after treatment with SCPs. **(H)** Image of the fluorescence intensity in CF1553. **(I)** Effect of SCPs on the lifespan of *pept-1(lg601) X.* mutants. **(J)** Effect of SCPs on the lifespan of *old-1(mk1) II* mutants. **(K)** Effect of SCPs on the lifespan of RB1159[*tyr-3(ok1194) I.*] mutants. **(L)** Effect of SCPs on the lifespan of RB1985[*acox-1.5(ok2619) III.*] mutants. Different letters correspond to statistically significant differences ($p < 0.05$) between groups.

demonstrated that SCPs have exceptional resistance to oxidative stress. SCPs were able to increase the expression of the *sod-3* fluorescent protein and considerably reduce the lifespan of *old-1* null mutants as compared to the control group, but they had no effect on *pept-1* null mutants.

In addition, SCPs may delay aging through other pathways. According to research, TYR-2 and TYR-3 deficiency causes increased CEP-1 activation and germ cell death (41). Dauer pheromones or daumones, both of which were signal molecules that interrupted development and reproduction (dauer larvae) during unfavorable growth conditions in *C. elegans*. Acox-1 of nematodes was an essential component of daumone biosynthesis (42), and *acox-1.5* was an ortholog of human ACOX1 (acyl-CoA oxidase 1). In the current study, SCPs significantly up-regulated the expression of the *tyr-3*, *acox-1.5*, and extended the lifespan of the *tyr-3*, *acox-1.5* null mutants. Positive controls were used in the study, Met and NMN. Metformin regulated the insulin and IGF-1 signaling (43), which were similar effects that SCPs exerted on *C. elegans*. NMN is a widespread reduction in NAD^+ that is connected to all of the signs and symptoms of aging. Age-related illnesses will be postponed or even reversed by restoring NAD^+ levels (44). These results indicated that SCPs could extend the lifespan, improve the health span and enhance stress resistance possibly via the *daf-2*,*daf-16*,*sod-3*,*old-1*, and *pept-1* axis in *C. elegans*, and to our knowledge, biologically active peptides to be studied in depth on *pept-1* expression, *old-1* is a gene that effectively regulates aging. This study is a new direction for the research of bioactive peptides on the development of peptides from sea cucumber (*Acaudina leucoprocta*) and the development and application of dietary sea cucumber peptides in mechanism research. The insulin pathway was given a new direction in particular. However, the resource utilization of *Acaudina leucoprocta* still needs to be enhanced and research ideas need to be further developed.

Conclusion

In this study, we have shown that SCPs from *Acaudina leucoprocta* increased lifespan and motility. SCPs not only increase the resistance of *C. elegans* to stress (heat and oxidative stress) but also inhibit lipofuscin and ROS accumulation, upregulate SOD and CAT activities, and reduces MDA content in *C. elegans*. Nematodes pretreated with SCPs showed increased expression levels of anti-aging genes (*daf-16*, *sod-3*, *pept-1*, *old-1*, *tyr-3*, *acox-1.5*), and decreased expression of *daf-2*. More than that, SCPs promote the migration of DAF-16 into the nucleus. SCPs extend the lifespan of *daf-2 (e1370)*, and activate *sod-3* green fluorescent protein expression and SCPs had no significant effect on lifespan in *pept-1* null mutants and were able to significantly reduce the lifespan of *old-1* null mutants. SCPs prolong the lifespan of *tyr-3* and *acox-1.5* null mutants. In summary, these results suggest that SCPs could extend the lifespan and enhance antioxidant capacity via DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1 not TYR-3/ACOX-1.5 in *Caenorhabditis elegans*.

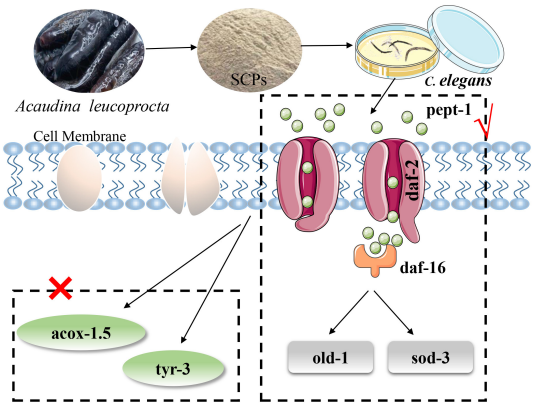

FIGURE 7
SCPs extended the lifespan of *C. elegans* could through the IIS pathway (*pept-1*, *daf-2*, *daf-16*, *old-1*, *sod-3*) but not apoptosis and Dauer phase.

Data availability statement

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

Author contributions

YW, JY, and CX designed experiments, carried out the experiments, analyzed the experimental results, prepared the original draft, and performed the statistical analysis. QL, YM, and SZ finished the validation. YW, JZ, FS, QW, and XZ

reviewed and edited the manuscript. XZ and FF acquired resources. 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.2022.1065145/full#supplementary-material

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Current status and frontier tracking of the China HACCP system

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In today's booming society and in the age of science and technology, the diversification of food processing methods, the continuous extension of the food trade chain, and the potential hazard factors in the food production process all make people pay more and more attention to the establishment, development, and improvement of the hazard analysis and critical control points (HACCP) system. Only terminal control and post-processing supervision of food can guarantee the absolute safety of food. In the process of processing, it is particularly important to strictly identify and evaluate the food safety hazards. To better assist food production enterprises in establishing and implementing HACCP systems, to implement the primary responsibility of food safety, and to improve the theoretical level and practical application of HACCP system in China, an investigation of the current situation and development frontier of HACCP system in China was conducted. Based on the core journal database of China Knowledge Network, the Chinese Social Science Citation Index database, and the Chinese Science Citation Database as the literature search database platform, the study used the CiteSpace visual metrics software system to analyze 1,084 pieces of literature in the field of HACCP research, in order to track the dynamics and impact of research in this field by Chinese research institutions and major authors, and analyze the research hotspots in the field. It is important for further research on HACCP. The results of the study showed that (1) the number of publications in the field of HACCP in China increased steadily from 1992 to 2004 and then began to decrease; (2) the indexes of journals with more publications were more concentrated, and the journal Food Science published the most; (3) the indexes of major research institutions showed that the cultivation bases of the State Key Laboratory of Chinese Medicinal Materials in the Center of Chinese Medicine Resources of the Chinese Academy of Traditional Medicine, the Guangdong Institute of Occupational Diseases, the Nanchang University of Life Sciences, and the Guangdong Institute of Occupational Diseases were more concentrated. Prevention and Treatment Institute, School of Life Sciences of Nanchang University, China Aquatic Products Quality Certification Center, School of Food Science and Nutrition Engineering of China Agricultural University, and other research structures have the most publications and strong scientific research strength; (4) from the main author indicators, the research in the field of HACCP has formed a total of four more active research teams, involving Chinese herbal medicine, ecological planting, ecological agriculture, occupational disease prevention and treatment, light industry handicrafts, computer software and computer application, agricultural economy, and other research directions. The cooperation between the authors of each team is closer. It is suggested that in terms of food safety requirements, China should not only integrate the traditional supervision measures for food terminals and after the event but also reflect

the role of food hazard analysis and assessment in the production process and comprehensively integrate the pre-production, production, and post-production management of food so that food can really be safe.

KEYWORDS

HACCP, food safety, process management, control and prevention, supervision and management, CNKI

1. Introduction

Modern industrialization and improving living standards have continuously increased people's expectations for food safety as compared to the past (1–3). The food production industry also paying greater attention to concepts like food safety control, prevention, and process management. In recent years, it has become widely recognized and accepted that the hazard analysis and critical control points (HACCP) system is capable of ensuring the safety of food (4–7). It is important not only to meet human needs, but also to improve the standards of food safety in social enterprises. It was determined that the search period was 1992–2022, and the source database was used for the search scope. A variety of databases were used to analyze the attention given to HACCP research, including journal databases, doctoral thesis databases, master's thesis databases, newspaper databases, and conference databases, as shown in Figure 1.

From Figure 1, it is evident that the attention of HACCP research has been on the rise since 1992, reaching its peak in 2004 with 563 research topics appearing during the year. During the period 1992 through 1997, China just stepped into the introduction phase of the HACCP system and received scant attention. In 1997, China sent a five-member expert group to participate in the first HACCP management teacher training course held by the food and drug administration (FDA) of the United States. Chinese HACCP research has been launched as a result of this study (8). In the period following 2000, researchers have been paying increasing attention to HACCP research. In 2001, China's first HACCP certification body "China Commodity Inspection Corporation HACCP Certification Coordination Center" was established. As of 2002, China's first special HACCP administrative rules were issued titled "Food production Enterprise Hazard Analysis and Critical Control Point (HACCP) Management System Certification Management Provisions." During a period of time in 2004, the Certification and Accreditation Administration of China compiled six textbooks about the HACCP system, held 37 training courses, and guided more than 4,000 export food production enterprises in six categories, who all established and implemented the HACCP system, thus igniting the interest of HACCP research. After 2004, however, the attention of HACCP research slowly subsided, with minor fluctuations in the process, which has been attributed to China's release of many standards related to food safety management systems. This reduction in attention may also be explained by the fact that the HACCP system gradually developed into a stable system.

Based on the China national knowledge infrastructure (CNKI) database as the data source, bibliometric analysis methods and literature visualization tools will be used to conduct the study. This study presents the research status, research hotspots, and development trends of China's HACCP system using a knowledge graph, in order to serve as a useful reference for subsequent research in this field. The bibliometric analysis method was chosen because it has been widely used in quantitative literature research in a particular subject area (9–14) and CiteSpace software is also an influential visual image analysis tool that can effectively provide a comprehensive assessment of a discipline or research

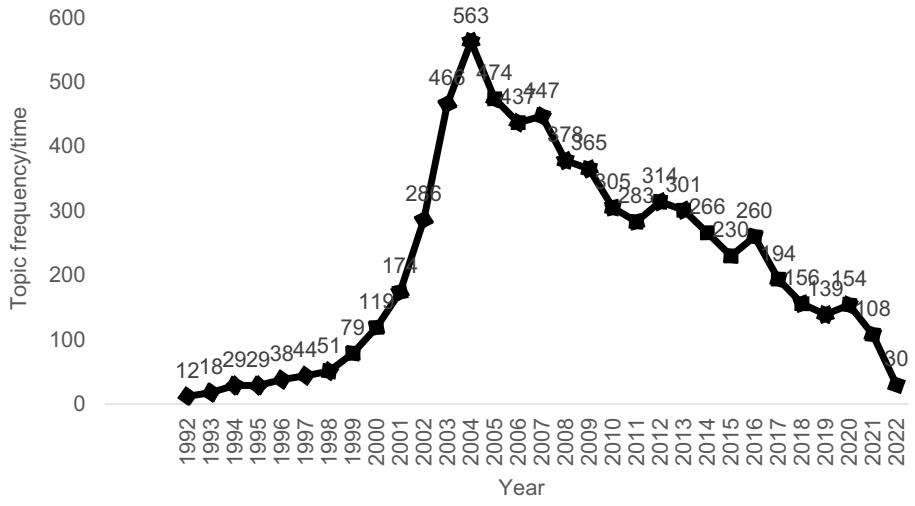

FIGURE 1
Research attention in the field of hazard analysis and critical control points (HACCP) from 1992 to 2022.

area (15–18). In addition, knowledge graphs can combine information visualization technology with traditional bibliometric analysis, and generate different types of knowledge graphs by integrating data mining, information processing, scientific measurement, and graph rendering to make the information more intuitive to researchers (19–23). This paper uses the methods of keyword clustering, keyword emergence and keyword periodic change of CiteSpace software to analyze and reveal the research hotspots, development ideas and strategic considerations in the field of HACCP in the future. The research results can not only improve the GMP, SSOP, GAP, and other food safety management systems but also empirically promote the "China HACCP system" to the world.

2. Materials and methods

2.1. Data sources

To ensure the authoritativeness and typicality of the research results, the following three databases in the CNKI were used: the Chinese social sciences citation index (CSSCI) Database, the Chinese science citation database (CSCD), and Peking University's core database. The scope of the selected and analyzed literature was limited to literature written in Chinese or published in China, resulting in the absence of research papers written by Chinese scholars but published in international journal platforms, which may have some impact on the rigor of the arguments and the importance of the findings, but the study still has some significance. First of all, the CSSCI, CSCD, and Peking University (PKU) core databases are important indicators of whether Chinese scholars, research institutions, and projects have completed their research. Further, although research papers written by Chinese scholars are published in international journals, similar "shadows" can also be found in domestic journals, which will not have a significant impact on the findings and conclusions of this study. As a result of the above two points, the significance and importance of this study will not be diminished.

In the study, "HACCP" was selected as the theme of accurate retrieval, and the retrieval period covered the period was 1992–2022. By conducting a preliminary search, 1,271 articles were obtained in total. Based on this, an in-depth manual interpretation of the titles and abstracts of the literature was conducted, to remove irrelevant literature, conference announcements, and news publicity, and a total of 1,084 books were retained in this study. As soon as the retrieved literature data was exported in Refworks format and saved to plain text files, CiteSpace software was used to convert and process the inherent data, thereby obtaining relevant data that can be considered as part of the data samples that were analyzed in this study. The paper conducted an accurate search after selecting a topic to avoid the possibility of a large number of irrelevant results. After the final determination of the sample size, the author exported and transcoded the target literature according to the reference format required by CiteSpace to obtain the research sample database for this paper.

2.2. Methods

2.2.1. CiteSpace software

CiteSpace is one of the most influential tools in bibliometric analysis (24–27). It is a Java application that analyzes and visualizes literature and is developed by Chen Chaomei, a Chinese scholar at Drexel University. It is possible to download CiteSpace software for free from the website linked to it.[1] It is the purpose of this paper to provide a comprehensive analysis of the research status and the latest advancements in the field of HACCP in China, based on the use of CiteSpace 5.8R3C version, in addition to the application of Excel.

In CiteSpace software, the analysis of collaboration networks (main authors and research institutions), co-occurrence networks (keywords), and other statistical analysis functions provides scholars with an objective assessment of the current state of the target research field in terms of time, research institutions, and members, as well as keywords (28–30). This paper adjusts and sets the parameters of CiteSpace software according to the requirements of these functions, based on existing research practices.

2.2.2. Parameter configuration

1. Time slicing was 1992–2022, and the year of each slice (Years Per Slice) = 1.
2. Node Types were, respectively, selected as research institution, author, and keyword.
3. Pruning Settings were selected as "Pruning sliced Networks".

3. Results

3.1. Examine the fundamental characteristics of the literature

The analysis of basic characteristics of literature primarily focuses on the quantity of published articles and the main sources of the journals. Among them, a change in the number of publications is an important indicator of measuring research within a specific field (31–33). Journals that mainly provide relevant literature in a certain field can be used to assess the authoritative journals in which researchers in that field are more likely to publish research.

3.1.1. Publication count

Based on the number of papers published in the field of HACCP, the overall data can be split into two stages: 1992–2004 has an upward trend, and 2004–2022 has a downward trend, as shown in Figure 2.

As can be seen in Figure 2, there was an upward trend in the number of published papers within the field of HACCP since 2002, with the number of published papers surpassing 60 for the first time in the past few years. In 2004, the number of articles reached 116, which marked the end of this upward trend. As of 2004, the number of published articles fluctuated up and down, but on the whole, the number of publications tended to decrease.

3.1.2. Primary journal sources

It can be seen in Figure 3 that the journals selected for publication are relatively concentrated in the field of HACCP research.

In the area of HACCP, Figure 3 showed that the top 10 journals were: "Food Science, ""Transactions of the Chinese Society of

1 http://cluster.ischool.drexel.edu/~cchen/CiteSpace/download/

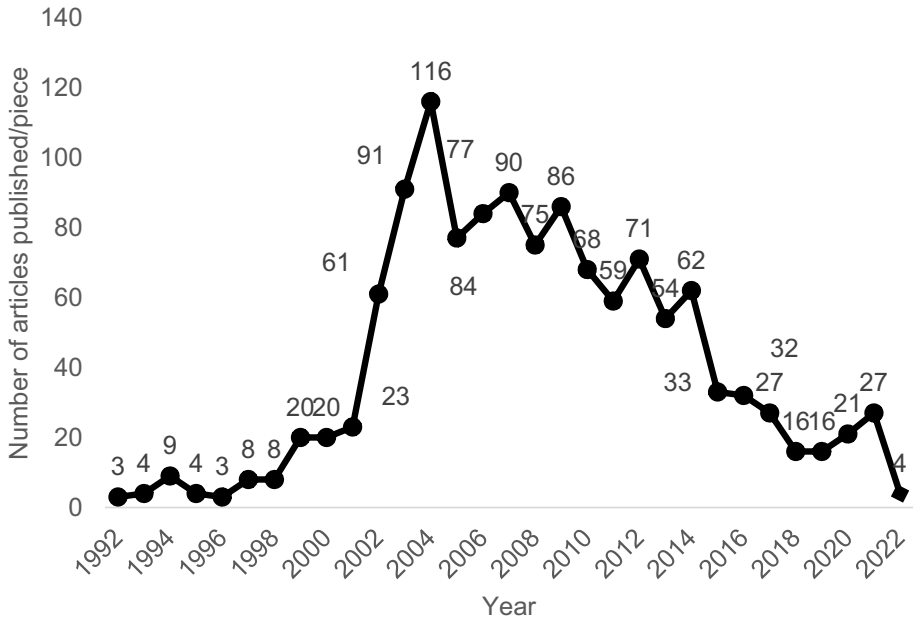

FIGURE 2
Quantity of publications in the field of hazard analysis and critical control points (HACCP).

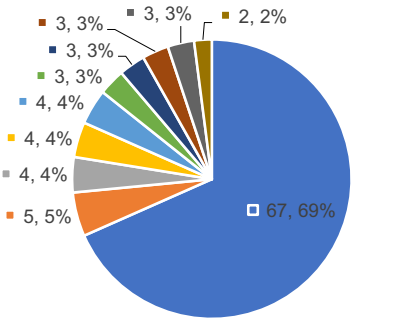

FIGURE 3
Main source journals in the field of hazard analysis and critical control points (HACCP).

Agricultural Engineering," "Issues in Agricultural Economy," "Journal of Agrotechnical Economics, ""Journal of Nuclear Agricultural Science," "China Journal of Chinese Materia Medica," "China Rural Survey," "Transactions of the Chinese Society of Agricultural Machinery," "China Soft Science," and "Chinese Traditional and Herbal Drugs." The largest number of articles were published in Food Science, accounting for 69% of the total number of articles published, with 67 articles in total. The number of articles published in other journals was relatively small, accounting for approximately 5% of the total. In light of this data, it appeared that Food Science will be the first choice for most HACCP studies to be published.

Based on Figure 4, the research disciplines in HACCP were relatively concentrated.

Figure 4 showed a relatively concentrated concentration of disciplines involved in the research and application of HACCP. These disciplines were ranked as the top 10 related disciplines: Light Industry Handicrafts, Macroeconomic Management and Sustainable Development, Industrial Economy, Agricultural Economy, Preventive Medicine and Hygiene, Animal Husbandry and Animal Medicine, Aquaculture and Fishery, Trade Economy, Horticulture, Computer Software and Computer Applications. The field of Light Industry Handicrafts accounted for the largest proportion, more than half, which indicated that the research and application of HACCP was most closely related to the discipline of Light Industry Handicrafts, followed by the discipline of Macroeconomic Management and Sustainable Development, accounting for 14%. Other subjects made up a relatively small proportion of the sample. Research conducted in the HACCP field is generally multidisciplinary, involved strong intersections, and encompassed a wide range of applications; most industries were closely related to the development of HACCP.

3.2. Examination of major research institutions and authors

The analysis of the main research institutions and authors in the field of HACCP research allows us to ascertain the main research forces and the levels of cooperation among the researchers. The

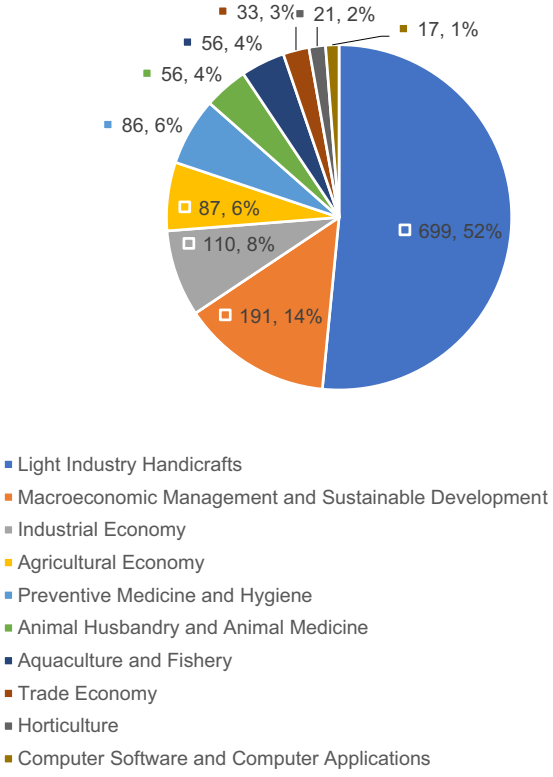

FIGURE 4
Top 10 related disciplines in the field of hazard analysis and critical control points (HACCP).

visualization map generated by CiteSpace software consists of nodes and lines, where nodes represent major research institutions, main authors, keywords, etc. The larger the node size, the more literature that has been published by the corresponding research institution or author; connections between nodes indicate collaboration between researchers and authors. Generally speaking, the thicker the connection, the closer the cooperation will be. As opposed to this, if there are no connections between nodes, it indicates the absence of a cooperative relationship between authors and organizations.

3.2.1. Main research institutions

A CiteSpace application was run with a time span of 1992–2022 set, a time slice of 1 year chosen, the node type selected as institution, and finally the data was compiled as follows: there were 498 nodes, there was one connection, there was zero network density, and the number of connections was one. It was important to note that the number of nodes represents the number of research institutions, and the connections between nodes represent some of those institutions appearing in the same literature; that was, they shared a cooperative relationship. Network density referred to the actual number of relationships divided by the maximum number of relationships in theory. It should be noted that there was no specific standard for this index. The data showed that there were many research institutions involved in HACCP, but the quantity of cross-institution collaborative research was relatively low. The main research institutions in the field of HACCP were showed in Table 1.

Based on the relevant data presented in Tables 1, a total of 14 research institutions published more than three articles related to the field of HACCP. Among them, the base of the State Key Laboratory of Authentic Medicinal Materials of Chinese Medicine Resource Center of the China Academy of Chinese Medical Sciences published the first and most articles, with a total of 32 articles in total. Guangdong Hospital for Occupational Disease Prevention and Control, the College of Life Science of Nanchang University, China Aquatic Product Quality Certification Center, and the College of Food Science and Nutritional Engineering of China Agricultural University were the other institutions with highest scientific research strength, all of which published five articles in the past year.

3.2.2. Primary authors

Node types in CiteSpace are set to author, and other parameters were set to the same as those in the research institution. A diagram of the lead author collaboration network in the HACCP domain could be found in Figure 5.

As can be seen from Figure 5, the main author collaboration network in the HACCP field was composed of 644 authors and 467 collaboration links, with a density of 0.0023. Based on these data, it can be seen that a large number of scholars were involved in the research area of HACCP and that there was a significant level of collaboration between researchers. For a more detailed examination of the number of papers published by the main authors in the field of HACCP and the collaboration between teams, the relevant data were summarized in Table 2.

The following were the main four teams related to HACCP field research, as demonstrated in Figure 5 and Table 2.

1. The research team anchored by Wan Xiufu published the largest number of papers, 32 in total, and the team members maintain frequent and close cooperation. This team primarily focuses on Chinese medicinal materials (34–38), ecological planting (39–41), ecological agriculture (42, 43).
2. The research team with Liu Ming as the core (a lot of the orange circle area) published nine articles. There was a close relationship between Liu Ming, Li Rongzong and Su Shibiao within the team, as well as a greater likelihood of cooperation between them. Liu Ming's research team focuses mainly on the study of HACCP (44–47), occupational hazards (48–51), and risk assessment (52, 53).
3. The research team centered on Liu Chunquan (blue circle area) published five articles. In terms of the number of articles published, Liu Chunquan and Liu Chunju were the most prominent. There were three main research directions of the team: HACCP (6, 54–56), crops (57, 58), and Materia medica (59).
4. The research team led by Wang Kaiyi (black circle area) published five articles. The graph above showed Wang Kaiyi's name separately as he published five papers related to HACCP alone. There were three major research directions emphasized by Wang Kaiyi's team: computer software and computer applications (60, 61), agronomy (62) and agricultural economics (63, 64).

3.3. Analysis of research hotspots

Typically, keyword clustering is used to identify the research topics in a certain research field within a given period of time, while

TABLE 1 Main research institutions in the field of hazard analysis and critical control points (HACCP).

Quantity of papers/piece	Time of first publication/year	Main research institution
32	1992	Cultivation base of State Key Laboratory of Authentic Medicinal Materials, Chinese Medicine Resource Center, China Academy of Chinese Medical Sciences
5	2014	Guangdong Province Hospital for Occupational Disease Prevention and Treatment
5	2007	School of Life Sciences, Nanchang University
5	2002	China Aquatic Product Quality Certification Center
5	2003	College of Food Science & Nutritional Engineering, China Agricultural University
4	2002	School of Food Science and Engineering, South China University of Technology
4	2004	College of Veterinary Medicine, China Agricultural University
3	2016	Harbin University of Commerce
3	2001	Shanghai Animal Husbandry and Veterinary Station
3	2001	Qingdao Exit-Entry Inspection and Quarantine Bureau
3	2007	College of Food Science, Fujian Agriculture and Forestry University
3	2006	Institute of food quality safety and testing, Jiangsu Academy of Agricultural Sciences
3	2005	School of Food Science and light Industry, South China University of Technology
3	2012	School of Environmental & Safety Engineering, Changzhou University

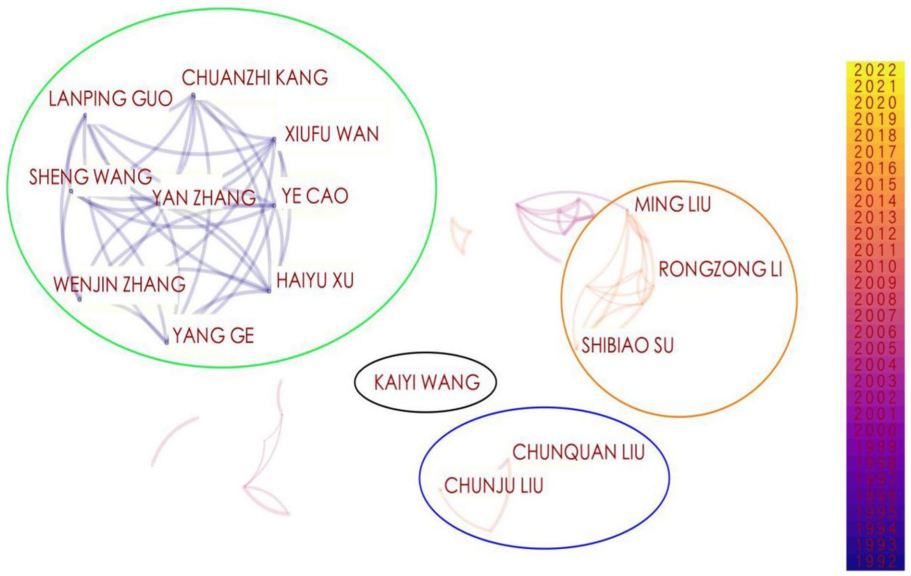

FIGURE 5
Hazard analysis and critical control points (HACCP) lead author collaboration network.

burst terms refer to the rapid increase or decrease in interest in a certain research field within a specific period of time. By detecting burst terms, research hotspots in different periods can be revealed (65).

3.3.1. Keyword clustering

For the purpose of in-depth exploration of the topic of HACCP research, the paper used CiteSpace software for keyword cluster analysis. Keyword cluster analysis referred to the use of clustering statistical methods based on co-occurrence analysis to simplify the occurrence network relationships into relatively small clusters. The node type was set as the keyword, and the settings of other parameters were the same as those of the research institution when the CiteSpace software is run. Based on the keyword knowledge network graph, the LLR algorithm was selected to obtain the HACCP research keyword clustering graph (Figure 6).

It can be seen from Figure 6 that the top 10 keyword clusters were hazard analysis, countermeasures, application, critical limit value, FDA, quality and safety, GMP, cross contamination, microorganism, and juice. Keyword cluster ranked from 0 to 9. The smaller the number, the more keywords were included in the cluster. A cluster consists of a number of closely related words. Which words were contained in each cluster? The keyword co-occurrence network clustering table (Table 3)

was obtained by using the log-likelihood algorithm (one of the clustering label word extraction algorithms). As indicated in the table, the silhouette represents a reasonable degree of clustering. It was generally believed that silhouette 0.5 indicated reasonable clustering results; silhouette 0.7 indicated satisfactory clustering results.

TABLE 2 The number of articles published by primary authors in hazard analysis and critical control points (HACCP).

Number	Quantity of papers/piece	Main author
1	32	Xiufu Wan
2	19	Chuanzhi Kang
3	19	Wenjin Zhang
4	18	Yan Zhang
5	17	Ye Cao
6	13	Sheng Wang
7	12	Haiyu Xu
8	12	Yang Ge
9	11	Lanping Guo
10	9	Ming Liu
11	8	Chunquan Liu
12	6	Rongzong Li
13	6	Shibiao Su
14	5	Kaiyi Wang
15	5	Chunju Liu

According to Table 3, the silhouettes of the top 10 clustering keywords in the HACCP field research were all greater than 0.7, which indicated that the clustering results were compelling, which also confirms the correctness of selecting HACCP as the title of this paper. For example, cluster #0, the topic of hazard analysis, mainly includes hazard analysis, critical control points, food safety, application, application research, and other keywords. These keywords were all related to the topic of food safety. The topics under the other cluster tags were generally the same as those under the first cluster tag.

According to Table 3, topics within each cluster have overlapping phenomena. In light of this, the related research in the field of Chinese HACCP can generally be categorized into two areas: "hazard analysis" and "process management."

3.3.1.1. Hazard analysis

Hazard analysis. The HACCP food safety system was a scientific and preventative system for identifying and controlling potential hazards, with the objective of minimizing the degree of harm. In hazard analysis, each link of a food production process was evaluated; the type of hazard is identified; the level of hazard was divided, the magnitude of hazard was analyzed; the significance of the hazard can be determined, critical control points were established, and a critical limit value was determined. Hazard analysis played an important role as a basis for process management, control, and prevention.

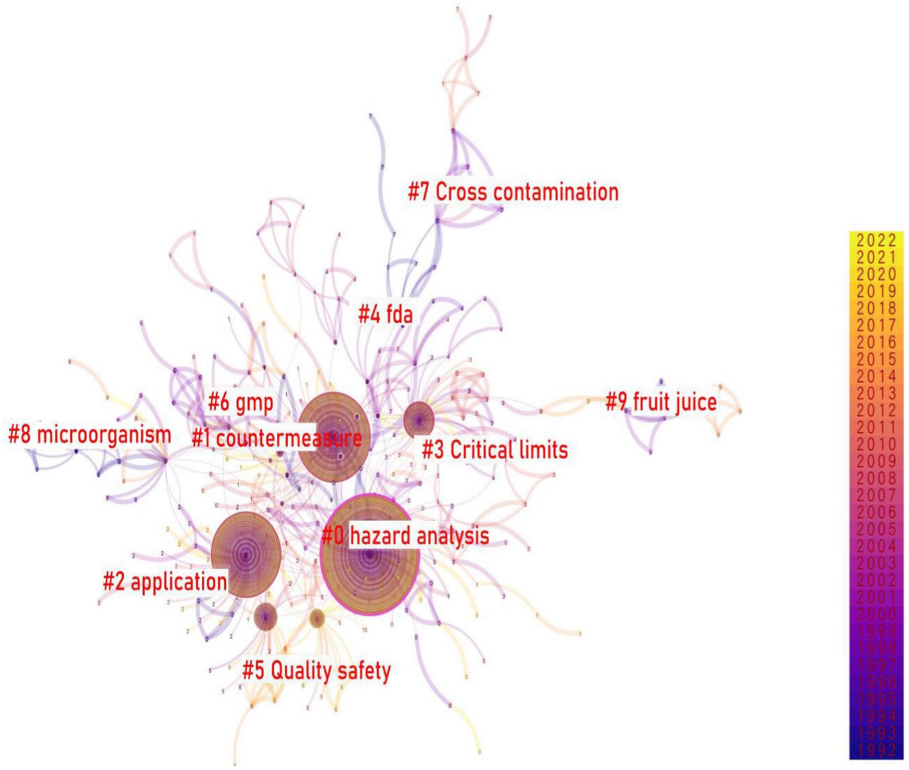

FIGURE 6
Hazard analysis and critical control points (HACCP) keyword clustering diagram.

TABLE 3 Clustering of keyword co-occurrence networks.

No.	silhouette	Cluster	Key words
#0	0.905	Hazard analysis	Hazard analysis; critical control point; food safety; application; application research on
#1	0.930	Countermeasures	Countermeasures; GAP; GACP; food safety; hazard analysis
#2	0.866	Application	Application; security; enterprise management; microcapsules; hazard analysis critical control point (HACCP)
#3	0.934	Critical limit value	Critical limit value; pond culture; compound feed; breeding base; pollution-free food
#4	0.956	FDA	FDA ; ISO ; feed industry; Canada; The management system
#5	0.938	Quality safety	Quality safety; quality control; agricultural products; vegetables; the supply chain
#6	0.915	GMP	GMP ; health supervision; SSOP. Quality of hygiene; raw milk
#7	0.959	Cross contamination	Cross contamination; harm; Salmonella; safety and health; the slaughterhouse
#8	0.985	Microbes	Microbes; Canned food; system; sterilization formula; Canned food sterilization
#9	0.980	Fruit juice	Fruit juice; real time control; the key point; safe water supply; small towns

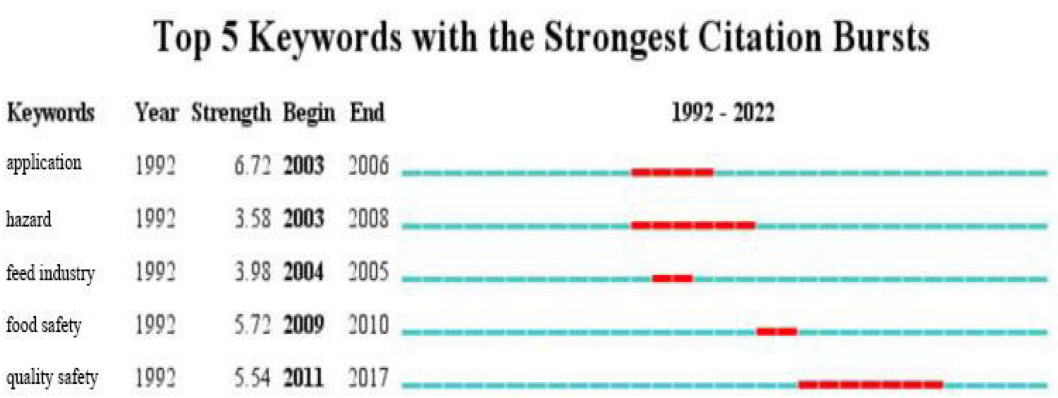

FIGURE 7
Keyword burst in the field of hazard analysis and critical control points (HACCP) research.

3.3.1.2. Process management

Process management. Following the establishment of the critical control point, the critical limit value should be determined, and the production process should be regulated in order to improve the quality and safety of the product. Under the premise of strict control of temperature, humidity, hygiene, time, and other factors, modern production equipment should be selected as far as possible in the process of food production, and monitoring methods and frequencies should be set to monitor each link of the production process. Hazard correction measures should be determined, and the whole production process should be recorded. Food safety control and prevention work can be implemented by strictly managing the production process.

3.3.2. Keyword burst

In CiteSpace software, the burst detection feature was primarily utilized to identify rapid changes in the number of references for a certain topic (66). A burst detection was viewed as an indicator of a highly active area of research that can be used to examine trends in emerging topics as well as provide a visual representation of the duration of these hotspots of research. The higher the intensity of keyword emergence, the more obvious the research orientation was, and the more it was a node of attention in a certain field of study.

As shown in Figure 7, the top five keywords with burst intensity from 1992 to 2022 were identified using CiteSpace software's burst detection function. As showed in the diagram, strength represents the intensity of the burst, begin represents the year during which bursts began, end represents the year when bursts ended, and the red part indicates the continuous burst throughout the year.

According to Figure 7, during the period 1992–2022, the keyword "application" was the highest burst intensity, with a strength of 6.72, and the start was 2003 and the end was 2006. Further, there were two keywords with a strength value greater than 5, namely "food safety" and "quality safety," indicating that they had always been concerned, particularly for "quality safety." Last but not least, the keyword with a strength value close to 4 was "feed industry," which had a short emergence time and does not receive sustained attention. Although it had not been popular for a long time, the keyword "harm," whose strength value was 3.58, was an unavoidable topic in all walks of life and had been addressed by all fields of society. Therefore, it was likely that the keyword "harm" will continue to be prominent in the future.

4. Discussion

Based on the analysis of the basic characteristics, main research institutions, main authors, and research hotspots in the field of HACCP in China, this paper discusses the research progress of HACCP and the research frontiers in the future. First of all, the study only counted and analyzed the number of documents issued in the HACCP field but did not count and analyze the cited quantity, which is one of the

disadvantages of CiteSpace software. Later, we will continue to explore the international food safety management system. Secondly, in the future, the research focus in the field of HACCP in China will still be on hazards analysis, countermeasures, applications, quality safety, GMP, and other topics. In China, the research focus has shifted from hazard analysis to the concept of hazard analysis integrated process management, with the realization that HACCP is a strategic consideration of integrated management and is a line management from pre-production, in-process, and post-production of food. There are also challenges to be faced.

In a word, the HACCP system is an internationally recognized and accepted food safety assurance system that meets the human requirements for food safety and also improves the social requirements for food safety standards in production enterprises. The study not only improved the previous food safety management systems such as GMP, SSOP, and GAP but also promoted the "China HACCP system" to the world from experience.

5. Conclusion

This study used CSSCI, CSCD, and the core of Peking University as sample data sources, based on the bibliometric analysis method and CiteSpace visualization software to produce a knowledge map of research in the field of HACCP in China from 1992 to 2022, and made a systematic and detailed analysis of the basic characteristics of the literature, main research institutions and authors, and research hotspots.

5.1. Fundamental characteristics of the literature

Fundamental characteristics of the literature. In terms of the number of publications, there were two main trends: an upward trend between 1992 and 2004, and a downward trend between 2004 and 2022. As for the main journal sources, the journals chosen to publish research articles in the field of HACCP tend to be more concentrated, such as the journal "Food Science," which had the most publications in the field. There was only a small proportion of articles published in other journals, and the number of articles published in each journal accounts for approximately 5% of the total number of articles published in the journal. According to this study, most HACCP researchers choose "Food Science" as their first choice. Furthermore, as far as related disciplines were concerned, the disciplines involved in the field of HACCP were also relatively specialized, among which the light industry and handicraft disciplines account for more than half of the total. This indicates that a much larger proportion of the research and applications for HACCP were focused on light industry and handicraft. Secondly, HACCP was closely related to macroeconomic management and sustainable development discipline. To sum up, HACCP field research involved multi-disciplines, strong intersections, and a wide range of applications.

5.2. The most important research institutions and authors

The most important research institutions and authors. As far as main research institutions were concerned, there was an abundance of research in the field of HACCP, but there was very little cross-institutional collaboration. The first and most of the papers published by the Chinese Medicine Resource Center, China Academy of Chinese Medical Sciences, are those published by the Cultivation base of the State Key Laboratory of Authentic Medicinal Materials. In terms of main authors, four active research teams had been formed in the field of HACCP, with Wan Xiufu, Liu Ming, Liu Chunquan, and Wang Kaiyi as the core, respectively. The main research directions included HACCP, Chinese medicine, ecological planting, ecological agriculture, occupational hazards, risk assessment, crops, computer software and computer applications, agronomy, agricultural economy, etc. The authors on each team collaborated closely.

5.3. Research hotspots

Research hotspots. As for keyword clustering, if the silhouette was greater than 0.7 after screening keywords related to HACCP field research, this indicated that the clustering results were satisfactory, ensuring the correctness of using HACCP as the title of the article. Further, there were overlapping phenomena among the research topics under each cluster, and related research in the Chinese HACCP field can be categorized into two areas: "hazard analysis" and "process management." From 1992 to 2022, the keyword "application" had the highest burst intensity, followed by "food safety" and "quality safety," indicating that these three topics had always attracted attention. In spite of the fact that the keyword "hazard" had not burst for a long time, the research related to this topic has always attracted the attention of all areas of society, and it had become an unavoidable topic in all walks of life. Therefore, the burst may continue to come into play for a longer period of time.

Author contributions

XS, XZ: conceptualization, data curation, and writing—original draft, XS, XZ, RA,TW, JZ, and YL: formal analysis, XS: funding acquisition, methodology; XS, TW: supervision, XS, XZ, and RA: writing—review and editing. 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

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Optimization of spray dried yogurt and its application to prepare functional cookies

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Introduction: Spray-dried yogurt powder (SDYP) has shelf stability and other functional properties that improve solubility and facilitate the use, processing, packaging, and transportation of other food derivatives, such as bread and pastries on a large scale. The present research was conducted to develop SDYP and further its utilization to prepare functional cookies.

Methods: Yogurt was spray-dried by employing different outlet air temperatures (OAT) (65°C, 70°C & 75°C) and inlet air temperature (IAT) (150°C, 155°C & 160°C). Spray drying shows that increasing the temperature increases nutritional loss, whereas *S. thermophilus* culture shows resistance to the intensive heat approaches. On the other hand *L. delbrueckii* subsp. Bulgaricus culture was found to be significantly affected. A total of 4 treatments, including one control for the functional cookies development.

Results and discussion: A directly proportional relation was investigated between the increasing concentration of SDYP and baking characteristics and cookie's mineral and protein profile. Bioactive parameters like antioxidant activity of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and total phenolic content (TPC) were also affected significantly. The sensory profile shows an incline towards T0 (0% SDYP) to T3 (10% SDYP) in all attributes but starts to decline when the concentration of SDYP reaches 15%. This study suggests that by employing a certain combination of temperatures (OAT: 60°C IAT: 150°C); maximum survival of inoculated culture can be achieved, and this powder can be utilized in the development of functional cookies with enhanced sensory as well as biochemical characteristics significantly ($P < 0.05$).

KEYWORDS

spray drying, yogurt, polyphenols, optimization, functional cookies

1. Introduction

The term "yogurt" originates from the Turkish word "yogurmak" which is literally defined as coagulating, thickening or cuddling. For a millennium, yogurt has been a part of the human diet around the globe (1). The health benefits of yogurt go back to 6,000 BC; in the 20th century *Stamen Grigorov* a Bulgarian medical student reported on the health-promoting aspects and advantages of lactic acid bacteria (2, 3). Because yogurt is a good source of protein with excellent bioavailability, a rich source of calcium, and a source of a variety of health-promoting probiotics, low yogurt consumption deprives you of the opportunity to contribute to a healthier lifestyle (4–6).

The quantity of viable cells in yogurt powder is a valuable indicator for determining the severity of heat damage caused during drying and optimizing processing settings (7–9). Powder items must satisfy the requirement that they have more than 10^6 colony-forming units (CFU)/ g at the expiration date to be considered healthful (7, 10). Because it has a high moisture removal rate, is less expensive, and takes less time to complete, spray drying is the most common and extensively researched alternative to freeze drying. It may be used for high throughputs and enables the preparation of stable and functioning products (11). The main issue with spray drying yogurt is keeping the lactic acid bacteria alive during and after drying (12). Spray drying must be light enough to prevent harming the heat-sensitive lactic acid bacteria while being effective enough to produce a powder with a moisture content below 4%, which is necessary for storage stability (13). Operating parameters for spray drying are primarily dependent on the equipment and the substance (14). The small range of potential operational parameters should be considered while optimizing spray-drying settings (15, 16).

The temperatures of the air entering and leaving the spray drier, the kind of atomization, and the airflow direction all impact the survival of yogurt bacteria. *Streptococcus thermophilus* was shown to have a greater survival rate than *Lactobacillus bulgaricus* during the spray drying of plain yogurt (17). However, both bacteria had comparable survival rates after the freezing process. The output temperature ranges of 70–75°C were ideal for *L. bulgaricus* and *S. thermophilus* survival; at these temperatures, the dried product's ultimate moisture content ranged from 5.1 to 6.3% (18). Therefore, the outlet air temperature during spray drying also significantly impacts the moisture content, color, and sensory characteristics of yogurt powder (19).

According to reports, fermented milk products have a hypocholesterolemic effect. Large amounts of fermented milk are recommended to offer factors that reduce cholesterol synthesis (20). *Lactobacillus acidophilus* has been reported to have the ability to reduce plasma cholesterol levels. Meanwhile, hypercholesterolemia is considered one of the leading causes of cardiovascular disease that can be treated with fermented dairy derivatives (20, 21). Dairy products have always been essential for human nutrition (18). However, further study of the product's anti-mutagenic, anti-cancer (22), and cholesterol-lowering properties will provide even greater opportunities for cultured dairy products, which are an essential component of human nutrition (23). However, to achieve the desired consumer benefits, it will be necessary to carefully select certain strains and combine them with appropriate production and processing procedures (24–26). Generally, probiotics and fermented foods are encouraged (27, 28).

Spray-dried milk powder has not only shelf stability but also other properties that improve solubility and facilitate the use, processing addition to packaging and transportation of other food derivatives such as bread and pastries on a large scale (29–32).

Cookies are one of the bakery products that all age groups people consume. These are usually prepared from refined wheat flour, which tends to have fewer essential nutrients but is a good source of fat, and carbohydrate (33, 34). The nutritional value can be enriched by dried yogurt powder, which adds protein, probiotics and minerals. The protein content of cookies can be increased by using yogurt powder (33, 35).

Spray drying can be employed for the drying of yogurt along with the highest survival of health-beneficial bacterial strains (36–38). SDYP can be an active functional ingredient for developing health-promoting food derivatives, which helps reduce health-related complications such as obesity. To develop SDYP, optimized processing conditions should be employed to achieve a certain derivative with acceptable probiotic bacteria viable count as well as maximum levels of protein content. There is no sufficient data available concerning the effect of spray drying on yogurt's physicochemical profile. However, yogurt powder is a great contender as a functional food constituent. It can be employed in developing functional cookies to enhance their nutritional value and bioactive profile. Taking all into context, the present study has been designed to evaluate the effect of drying yogurt by using the spray drying technique and further its application in developing functional cookies at different levels.

2. Materials and methods

2.1. Procurement of raw material

Raw material (i.e., milk, wheat flour, sugar and fat etc.) was purchased from the local market of Faisalabad city in the highest possible quality. A total of 1,500 mL milk sample was collected in 3 sterilized glass bottles with having capacity of 500 mL each. Particularly milk samples were stored in a cool box (5 ± 2°C). Regents required in this effort were directly purchased from the scientific store of the highest possible quality. The collected raw material was transferred to the "Hi-Tech labs" facility at Government College University Faisalabad for further examination or development.

2.2. Development of yogurt

Milk samples were pasteurized before the inoculation of starter culture by employing high-temperature–short-time (HTST) technique with some modifications. For this purpose, accurately 200 mL of milk sample was taken in a graduated conical flask container of 500 mL capacity and further placed into a water bath (Model-1235 PC, *VWR scientific*, Singapore) and the water bath was allowed to heat up until required temperature (72°C) is achieved.

TABLE 1 Temperature optimization for spray drying of yogurt.

Treatments	OAT (°C)	IAT (°C)	FT (°C)
T1	65	150	9
T2	70	155	9
T3	75	160	9

OAT, outlet air temperature; IAT, inlet air temperature; FT, feed temperature.

TABLE 2 Different levels of SDYP in cookies.

Treatments	Composition	
	Wheat flour (%)	SDYP (%)
T0	100%	0%
T1	95%	5%
T2	90%	10%
T3	85%	15%

SDYP, spray-dried yogurt powder.

After the pasteurization step, milk was placed in a glass jar, and starter culture comprising *L. bulgaricus* and *S. thermophilus* was introduced. The further fermentation process was carried out for about 8–12 h.

2.3. Spray drying of yogurt

Yogurt was stirred in a blender for 1 min (1st 20 s at low speed; 2nd 20 s high speed and 3rd 20 s again at low speed) and quickly heated up to the certain feed temperature before spray drying. Experiments were conducted in a pilot-scale spray dryer. Stirred yogurt was atomized from the nozzle into a vertical co-current drying chamber, 0.87 m diameter and 1.2 m in height, under various operating conditions. Atomizing air pressure of 296 kPa and hot air flow rate of 1.54 m^3/m were fixed for all experiments. Inlet air temperature (150–160)°C, outlet air temperature (65–75)°C, and feed temperature 9°C were in the range of and were adjusted according to the central composite rotatable design (CCRD; Table 1). The outlet air temperature was controlled by regulating the feeding velocity. The dried powder was collected in a single cyclone separator, packaged in glass jars, and kept in the dark until used for analysis.

2.4. SDYP analysis

2.4.1. Culture survival

To determine survival rate of the lactic acid bacteria, dried samples were rehydrated to the initial solids level of fresh yogurt with distilled water. *S. thermophilus* and *L. bulgaricus* was enumerated according to TS ISO 7889. Ten grams of reconstituted yogurt powder was mixed with Ringer's solution and decimal dilutions were prepared. For the enumeration of *S. thermophilus*, M-17 agar plates were incubated aerobically at 37°C for 48 h and the double-layer inoculated MRS plates for *L. bulgaricus* were incubated anaerobically at 37°C for 72 h. The average counts from the plates of 30–300 colonies were calculated and the results were expressed in cfu/g dry matter.

TABLE 3 Proximate chemical analysis of spray dried powder at different optimized conditions.

Trials	Physicochemical profile			
	Moisture content	Protein	Lactose	pH
T1	9.60 ± 0.03a	34.73 ± 0.35c	42.89 ± 0.19b	5.43 ± 0.05a
T2	6.33 ± 0.01b	35.79 ± 0.11b	44.71 ± 0.11a	5.40 ± 0.02b
T3	5.08 ± 0.02c	36.28 ± 0.27a	44.80 ± 0.03a	5.43 ± 0.06a
Average	7.00 ± 2.08	35.60 ± 0.74	44.14 ± 0.96	5.42 ± 0.04

T1, OAT 65°C: IAT 150°C: FT 9°C; T2, OAT 70°C: IAT 155°C: FT 9°C; T3, OAT75°C: IAT 160°C: FT 9°C.
Each value is an average of three observations.
Means that do not share a letter are significantly different at level ($P < 0.05$).

2.4.2. Moisture content

The moisture content of prepared cookies and dried apple pomace was used as prescribed by AOAC (39). Exactly, 2 g sample was taken into the crucible of known mass and placed into the oven for 3 h at 105°C. Further, the samples were cooled down in a desiccator and weighed using a digital balance. Obtained results were statistically analyzed and discussed in the fourth chapter. By the difference in mass, the mass of the moisture content in samples was calculated by the expression.

Calculations:

$$Moisture\ content = (W_2 - W_3)/(W_2 - W_1) \times 100$$

where:

W1, weight of empty crucible; W2, weight of crucible + sample before drying; W3, weight of crucible + sample after drying to constant mass.

2.4.3. Protein content in SDYP

The protein content of prepared SDYP was determined by utilizing Micro-Kjeldahl method as prescribed by AOAC (39). For this purpose, a conversion factor of 6.25 was employed to determine the total nitrogen. Accurately, 2 g of the sample was poured into the test tube, followed by 10 ml H_2SO_4 in the presence of selenium as a catalyst. However, digestion of the mixture continued until a clear solution appeared. After the digestion, the solution was transferred into a graded flask containing 100 mL of distilled water. Exactly, 10 mL of the prepared solution was mixed with equivalent volumes of NaOH (45%) solution and distilled by Kjeldahl apparatus. After distillation, the mixture was transferred into a 100 mL (4%) boric acid flask. Accurately, three drops of methyl red indicator and thoroughly mixed.

Moreover, 50 mL distillate was titrated against 0.02 N H_2SO_4 solution until the color changed from green to red. Obtained results were statistically analyzed. The expression calculated the nitrogen (%) content.

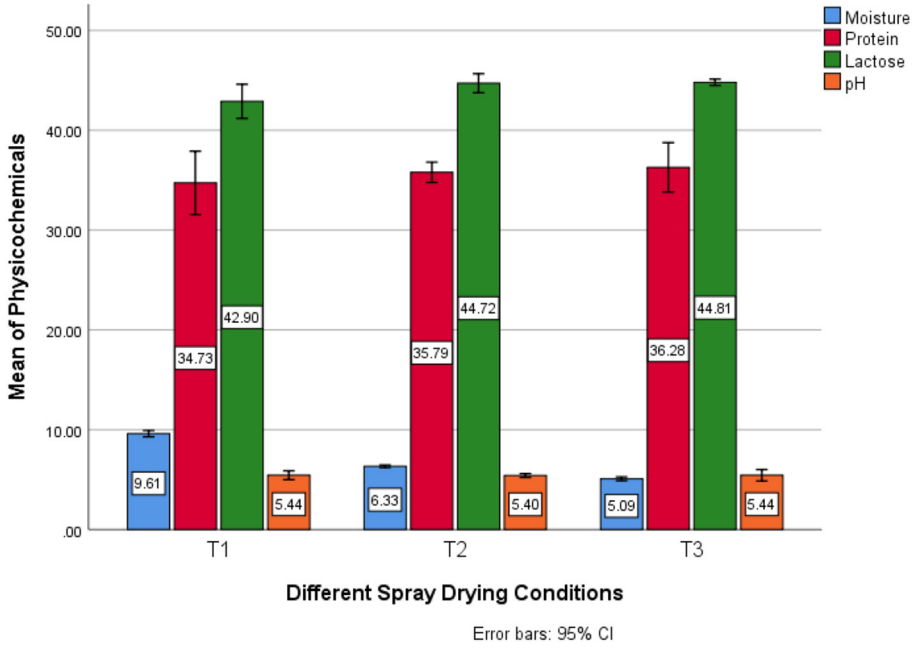

FIGURE 1
Estimated marginal means for physicochemical profile of SDYP.

Calculations:

$$Nitrogen\ (\%) = (V_s - V_B \times N_A \times 0.01401)/W \times 100$$

where: VS, volume (ml) of acid required to titrate sample; VB, volume (ml) of acid required to titrate blank; NA, normality of acid; W, weight of samples in grams.

$$\%\ crude\ protein\ =\ N2\ \times\ conversion\ factor$$
$$100\%\ nitrogen\ in\ protein\ =\ conversion\ factor$$
$$100/16 = 6.25$$

where: N2, nitrogen.

2.4.4. pH determination

After the SDYP was rehydrated, it was analyzed for pH determination through a method prescribed by AOAC (40). Sample was thoroughly mixed and 15 g sample was taken in a china dish. After that, the pH. and temperature electrodes were wiped out with tissue paper and then placed in china dish. Both the readings of time and temperature were noted from the digital pH meter display screen. This method mentioned above was repeated for all samples (Temperature). The obtained data were statistically analyzed and stated in the results section.

NOTE: After the above-mentioned analysis, the treatment with the least physicochemical loss and maximum survived (Culture) has been further employed in different concentrations to develop functional cookies.

TABLE 4 Effect of different spray drying parameters on culture survival.

Trials	Inoculated culture			
	A	Survival%	B	Survival%
Initial value	9.5×10^8	NA	9.5×10^8	NA
T1	5.8×10^8	61.1	1.7×10^8	17.9
T2	5.2×10^8	54.17	1.5×10^8	15.8
T3	4.9×10^8	51.6	1.3×10^8	13.7
Average	6.37×10^8		3.52×10^8	

T1, OAT 65°C: IAT 150°C: FT 9°C; T2, OAT 70°C: IAT 155°C: FT 9°C; T3, OAT75°C: IAT 160°C: FT 9°C; A, Streptococcus thermophilus (cfu/mL); B, Lactobacillus delbrueckii subsp. bulgaricus (cfu/mL); NA, not applicable.

2.5. Development functional cookies

After the preparation of SDYP, it was added at different levels in the preparation of cookies, as shown in Table 2. The preparation steps were as follows: the ingredients (the whole wheat flour and dried apple pomace) have been used in a bowl. Further, fat, milk and salt were mixed for 30 min using the rubbing method. Kneading of dough was done by adding egg and water into the flour-based mixture in a separate bowl. In the next step, the dough was rolled and flattened into the similar thickness of about 3.5 mm before cutting into shapes using a hand cutter. The cut dough was baked in the oven at 150°C for ~30 min. After baking, the cookies were allowed to cool down at ambient room temperature and then packed in vacuumed low-density polyethylene bags to avoid contamination until further analysis.

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TABLE 5 Effect of adding SDYP on the physicochemical profile of cookies.

Descriptives (g/100 g)						
Treatments	Moisture	Protein	Fat	Fiber	Ash	Nitrogen free extract (NFE)
T0	4.69 ± 0.26d	10.46 ± 0.01d	22.82 ± 0.03a	0.42 ± 0.03a	1.43 ± 0.01d	60.19 ± 0.19a
T1	4.96 ± 0.04c	12.09 ± 0.02c	22.14 ± 0.02b	0.41 ± 0.02a	1.44 ± 0.02c	58.96 ± 0.10b
T2	5.07 ± 0.04b	14.75 ± 0.04b	21.89 ± 0.04c	038 ± 0.04b	1.46 ± 0.01b	56.45 ± 0.12c
T3	5.20 ± 0.02a	16.19 ± 0.03a	20.98 ± 0.01d	0.35 ± 0.01c	1.48 ± 0.01a	55.80 ± 0.08d
Average	4.98 ± 0.22	13.37 ± 2.39	21.95 ± 0.70	0.39 ± 0.02	1.45 ± 0.02	57.85 ± 1.92

T0, control (100% wheat flour: 0% SDYP); T1, control (95% wheat flour: 5% SDYP); T2, control (90% wheat flour: 10% SDYP); T3, control (85% wheat flour: 15% SDYP).
Each Value is an average of three observations.
Means that do not share a letter are significantly different at level ($P < 0.05$).

TABLE 6 Effect of adding SDYP on the baking characteristics of cookies.

Descriptives				
Attributes				
Treatments	Diameter (mm)	Thickness (mm)	Spread ratio (D/T)	Color
T0	44.15 ± 0.01a	1.50 ± 0.01c	29.80 ± 0.03d	156.86 ± 0.55d
T1	44.17 ± 0.01a	1.52 ± 0.01c	30.20 ± 0.01c	165.16 ± 1.10c
T2	44.24 ± 0.01b	1.57 ± 0.01b	30.77 ± 0.02b	173.08 ± 0.44b
T3	43.37 ± 0.03c	1.64 ± 0.01a	31.09 ± 0.02a	180.97 ± 0.06a
Average	43.98 ± 0.38	1.55 ± 0.06	30.46 ± 0.53	169.02 ± 9.61

T0, control (100% wheat flour: 0% SDYP); T1, control (95% wheat flour: 5% SDYP); T2, control (90% wheat flour: 10% SDYP); T3, control (85% wheat flour: 15% SDYP).
Each Value is an average of three observations.
Means that do not share a letter are significantly different at level ($P < 0.05$).

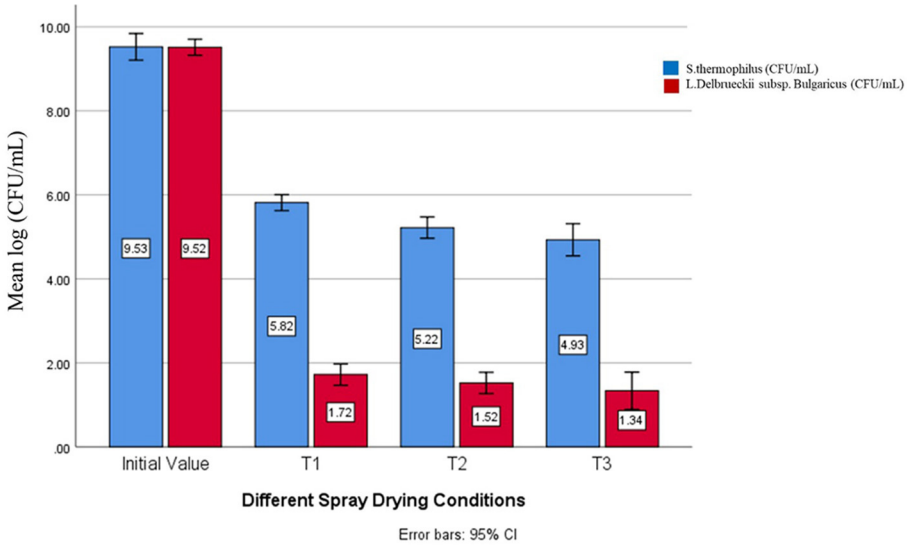

FIGURE 2
Estimated marginal means for culture survival in SDYP.

2.6. Proximate chemical analysis

Prepared SDYP and functional cookies samples were analyzed for their basic chemical profile i.e., moisture content, ash content, protein content, fat as well as fiber content and nitrogen free extract according to methods prescribed by AOAC (41). The calculations are as follows:

$$Moisture\ content = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

$$Ash\ Content\ (\%) = \frac{(W1)\ Weight\ of\ Ash}{(W)\ Weight\ of\ initial\ sample} \times \frac{100}{1}$$

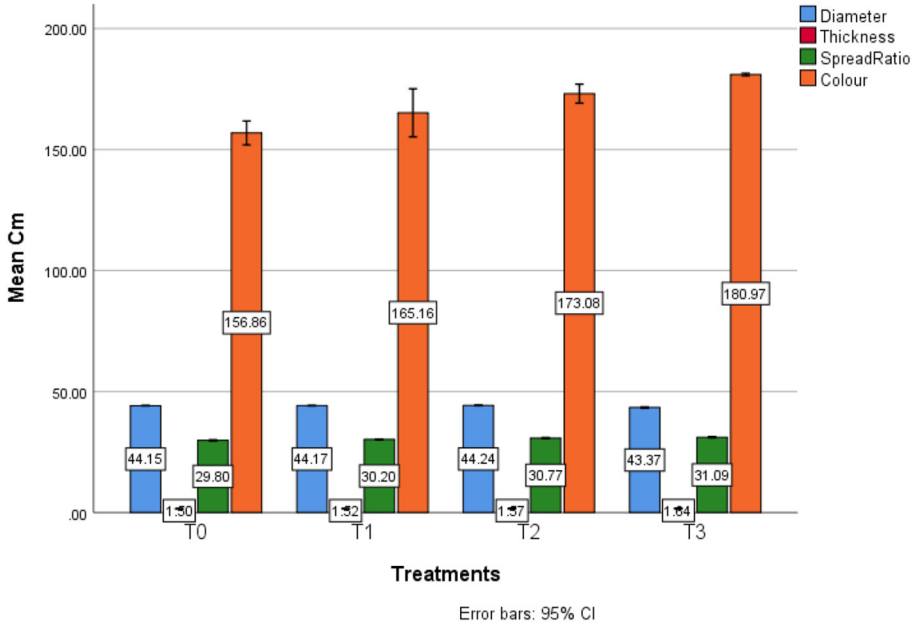

FIGURE 3
Estimated marginal means for baking profile in different treatments.

$$\frac{W_2 - W_1}{Weight\ of\ sample} \times 100$$

$$Nitrogen\ (\%) = \frac{V_s - V_B \times N_A \times 0.01401}{W} \times 100$$

100% Nitrogen in protein = conversion factor.

$$\frac{100}{16} = 6.25$$

$$Crude\ Fat\ (\%) = \frac{Mass\ of\ Fat}{Mass\ of\ Sample} \times 100$$

$$Crude\ Fiber\ (\%) = \frac{W_1 - W_2}{W_3} \times 100.$$

2.7. Bioactive profile of developed functional cookies

2.7.1. Extraction of soluble phenolic compounds

For this purpose, samples were ground into a fine powder using a KMF grinder at 9,676.8 × g. Prepared ground samples were kept in sterile bags to prevent contamination at −40°C until further extraction. Methyl alcohol, ethyl alcohol and water were used to prepare extracts. Accurately, 0.5 g of dried sample was added into a flask followed by exactly 100 mL ethyl acetate and stirred at 20°C for 3 h. The prepared mixture was kept in the dark to avoid any exposure to light for this purpose, aluminum foil was employed; the mixture was held for 12 h. Next, mixture was centrifuged at 9,676.8 g for 30°C. Further, concentrate was filtered by Whatman filter paper (No 1, Ø 155 mm). However, prepared extracts were stored at ∼4°C for until further analysis (42, 43).

2.7.2. Antioxidant activity (DPPH assay)

Free radical scavenging activity was examined by method (2, 2-diphenyl-1-picryl hydrazyl) as prescribed by Mphahlele et al. (44) with slight modifications as mentioned above. For this purpose, accurately 15 µl extracts were added into a test tube followed by 735 µl methanol and 750 µL 0.1 mM DPPH solution and thoroughly mixed until the extract dissolved in methanol. Then the mixture was incubated for precisely 30 min in the dark to avoid any exposure to light. The absorbance was measured at 517 nm by employing Ultra Violet visible spectrophotometer. A suitable calibration curve was prepared by using ascorbic acid as standard solution. The results were expressed as mM ascorbic acid (AA) equivalent g^{-1} of extracts.

2.7.3. Total phenolics content

In this study, prepared extracts were examined for their total phenolics content (TPC) by Folin-Ciocalteu method as prescribed by Al-Rawahi et al. (45). For this purpose, accurately 70 µL of prepared extracts were added in a test tube of 10 ml capacity, followed by 250 µl of Folin-Ciocalteu reagent and 750 µL of Na_2CO_3 (1.9 M). However, a total volume of exactly, 5 m was made up by adding distilled water and then mixed by using a vortex mixer for about 1 min prior to incubation for 2 h. in the dark. Consequently, the absorbance was measured using spectrophotometer (Thermo-Spectronic, Surrey, England) at

TABLE 7 Effect of adding SDYP powder on bioactive profile of cookies.

Descriptives			
Bioactive parameters			
Treatments	DPPH (%)	ABTS (%)	TPC (GAE mg/100 g)
T0	61.32 ± 0.04d	37.20 ± 0.01d	4.94 ± 0.01d
T1	67.26 ± 0.13c	50.24 ± 0.16c	7.26 ± 0.01c
T2	69.82 ± 0.13b	59.36 ± 0.05b	10.14 ± 0.03b
T3	72.75 ± 0.08a	77.95 ± 0.05a	16.01 ± 0.04a
Average	67.79 ± 4.50	56.18 ± 15.85	9.59 ± 4.42

T0, control (100% wheat flour: 0% SDYP); T1, control (95% wheat flour: 5% SDYP); T2, control (90% wheat flour: 10% SDYP); T3, control (85% wheat flour: 15% SDYP).
Each value is an average of three observations.
Means that do not share a letter are significantly different at level ($P < 0.05$).

765 nm wavelength. Calibration curve was prepared by employing controlled solutions of gallic acid. Obtained results were expressed as gallic acid equivalents (GAE) in mg^{-g} dry solids.

2.7.4. Total flavonoids content

Total flavonoids content (TFC) content of the extract was determined by a method as prescribed by Al-Rawahi et al. (45). For this purpose, exactly 1 mL of prepared extract was placed into a test tube (10 mL) already containing 4 mL of distilled water. At instant, 0.3 mL of 5% sodium nitrite was added into the test tube. However, after 5 min accurately, 0.3 mL of 10% Aluminum chloride was also placed in the same test tube. Then after 6 min exactly, 2 mL of 1 M sodium hydroxide was added to the test tube and mixed. Instantly, the test tube was diluted with the addition of 2.4 mL of distilled water and thoroughly mixed.

At last, the absorbance of the pink-colored mixture was examined at 510 nm and water was used as a blank. A suitable calibration curve was prepared to utilize different concentrations of catechin solutions. The results were mg catechin equivalent (CE)/g of dry solids.

2.8. Sensory evaluation

Sensory evaluation of functional cookie samples was done using a 9-point hedonic scale. Sensory attributes were judged by a panel of trained judges relevant to the field of study. The parameters on the scale were as follows: 1 = dislike, 2 = dislike slightly, 3 = neither like or dislike, 4 = like moderately, 5 = like very much, 6 = like extremely, 7 = good, 8 = very good, 9 = excellent. Scores given by the judges were statistically analyzed (descriptives and ANOVA).

2.9. Statistical analysis

The obtained results were subjected to the analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) Version 25 and the treatment means were separated using Fishers Less Significant difference (LSD) test.

3. Results

3.1. Effect of spray drying on the physicochemical profile of yogurt powder

As shown in Table 3, after the development of yogurt powder, samples were examined for their moisture; on average in T1 (9.60 ± 0.03) g/100 g, T2 (6.33 ± 0.01) g/100 g and in T3 (5.08 ± 0.02) g/100 g was found. Meanwhile, the highest water content was discovered in T1 (9.60) g/100 g samples treated with 65°C outlet temperature and 150°C of inlet air temperature. On the other hand, the lowest moisture content was investigated in T3 (5.08) samples being treated with 75°C outlet temperature and 160°C of inlet air temperature. Regarding the protein results, on average, in T1 (34.73 ± 0.35) g/100 g, T2 (35.79 ± 0.11) g/100 g and T3 (36.28 ± 0.27) g/100 g were found; meanwhile, the highest protein content was discovered in T3 (36.28) g/100 g samples treated with 75°C outlet temperature coupled with 160°C of inlet air temperature, on the other hand, least protein content was calculated in T1 (5.08) g/100 g samples being treated with 65°C outlet temperature coupled with 150°C of inlet air temperature. Interestingly, on average lactose value in T1 (42.89 ± 0.19) g/100 g, T2 (44.71 ± 0.11) g/100 g and in T3 (44.80 ± 0.03) g/100 g was found; meanwhile, the highest protein content was discovered in T3 (44.80) g/100 g samples treated with 75°C outlet temperature coupled with 160°C of inlet air temperature, on the other hand, least lactose content was calculated in T1 (42.89) g/100 g samples being treated with 65°C outlet temperature coupled with 150°C of inlet air temperature. As far as the pH determination was concerned it was clearly shown in Table 1. That on average, in T1 (5.43 ± 0.05), T2 (5.40 ± 0.02) and T3 (5.43 ± 0.06) were found; meanwhile, the highest pH value was defined in T3 (5.44) samples treated with 75°C outlet temperature coupled with 160°C of inlet air temperature whereas, minimum pH value was measured in T2 (5.40) samples being treated with 65°C outlet temperature coupled with 150°C of inlet air temperature (Figure 1).

3.2. Effect of different spray drying conditions on survival of inoculated culture

Two different strains were added for fermentations A: *S. thermophilus* and B: *Lactobacillus delbrueckii* subsp. *bulgaricus* in the current venture. Further, after each treatment (T0, T1, T2, and T3) yogurt powder was examined for both A and B strains which are depicted from (Table 6) on average in the initial sample 9.5 × 10^8 log cfu/mL was detected for both A and B strains as far the A culture reduction was concerned, on average in T1 (5.8 × 10^8) log cfu/mL (61% survival), T2 (5.2 × 10^8) log cfu/mL (54.17% survival) and in T3 (4.9 × 10^8) log cfu/mL (51.6% survival) was investigated. On the other hand, in culture B: on average in T1 (1.7 × 10^8) log cfu/mL (17.9% survival), T2 (1.5 × 10^8) log cfu/mL (15.8% survival) and in T3 (1.3 × 10^8) log cfu/mL (13.7% survival) was found as shown in Table 4 and Figure 2.

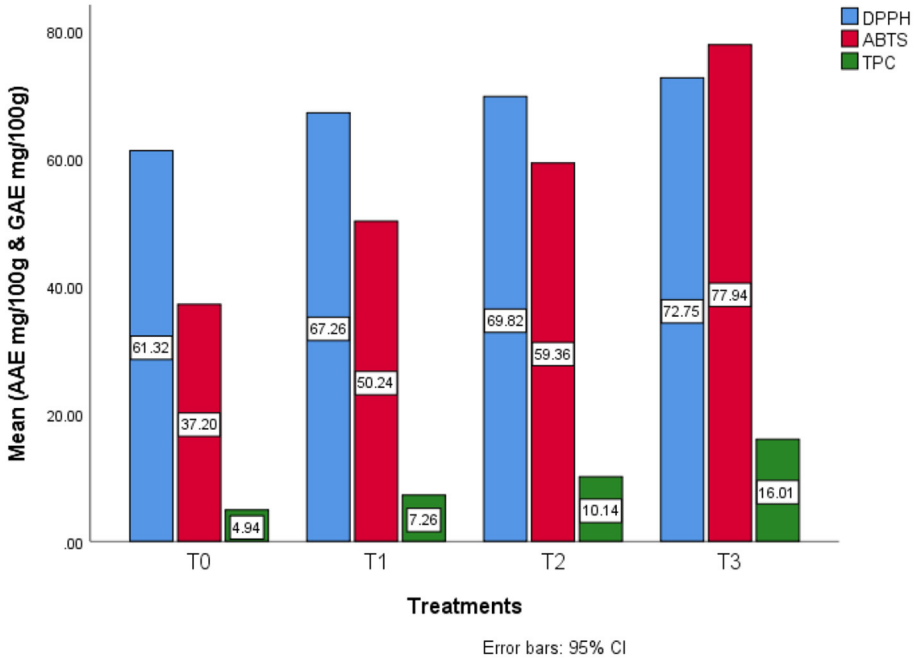

FIGURE 4
Estimated marginal means for bioactive profile in different treatments.

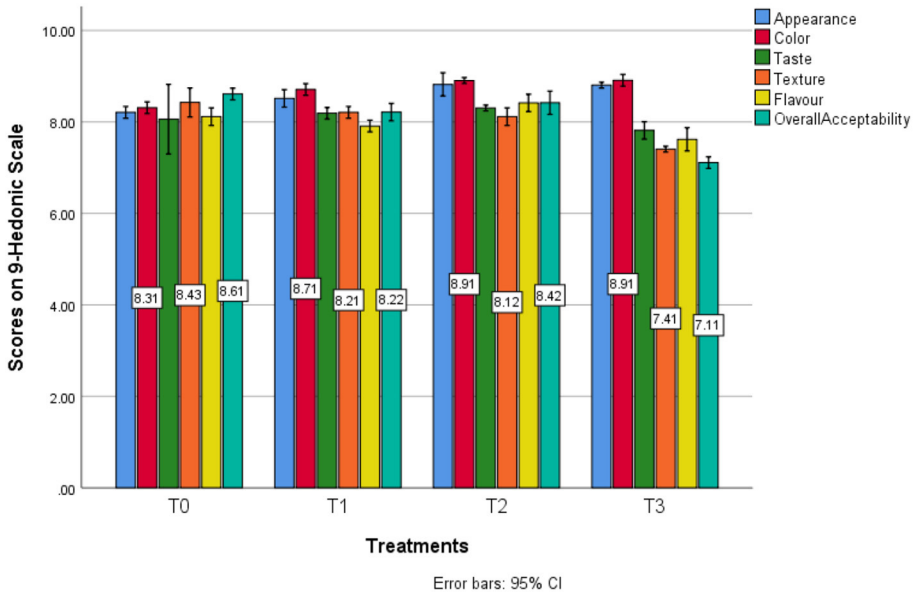

FIGURE 5
Estimated marginal means for sensory profile in different treatments.

3.3. Effect of adding SDYP on the proximate chemicals of functional cookies

After SDYP, cookies were prepared by partially substituting wheat flour with yogurt powder to enhance their bioactive profile, biochemical composition, and the desired sensory appeal. For this purpose, four treatments (T0: 0% SDYP, T1: 5% SDYP, T2: 10% SDYP and T3: 15% SDYP), including one control, were introduced in this study. However, the obtained results of its biochemical and bioactive and sensory profile were statistically analyzed as follows: Descriptive analysis of moisture content in different treatments was conducted. The maximum moisture content (5.21) g/100 g was detected in T3 sample with 15% SDYP whereas the least moisture level was examined in T0 (Control) g/100 g sample containing 0% SDYP. Examination shows that by adding SDYP moisture content of cookies significantly increased as compare to the cookies

TABLE 8 Effect of adding SDYP on the sensory profile of cookies.

Sample	Appearance	Color	Taste	Texture	Flavor	Overall acceptability
T0	8.2c	8.3c	8.0b	8.4a	8.1b	8.6a
T1	8.5b	8.7b	8.2a	8.2b	7.9c	8.2c
T2	8.7a	8.8a	8.3a	8.1b	8.4a	8.4b
T3	8.8a	8.9a	7.8c	7.4c	7.6d	7.1d

T0, control (100% wheat flour: 0% SDYP); T1, control (95% wheat flour: 5% SDYP); T2, control (90% wheat flour: 10% SDYP); T3, control (85% wheat flour: 15% SDYP).
Each value is an average of three observations.
Means that do not share a letter are significantly different at level ($P < 0.05$).

developed with only wheat flour and further found in following order (Wheat: SDYP) of T0 (100:0) < *T1* (95:05) < *T2* (90:10) < *T3* (75:15) at level ($P < 0.05$). Protein content in different treatments were conducted which clearly shows that the maximum protein content (16.22) g/100 g was detected in T3 sample having 15% SDYP whereas, least protein level (10.45) was examined in T0 (Control) g/100 g sample containing 0% SDYP. Analysis clearly shows that by adding SDYP protein content of cookies significantly increased as compare to the cookies developed with only wheat flour and further found in following order (Wheat: SDYP) of T0 (100:0) < *T1* (95:05) < *T2* (90:10) < *T3* (75:15) at level ($P < 0.05$). Fat content in different treatments was examined. Regarding the outcomes, determination shows that by adding SDYP fat content of cookies significantly decreased as compare to the cookies developed with only wheat flour and further found in following order (Wheat: SDYP) of T0 (100:0) > *T1* (95:05) > *T2* (90:10) > *T3* (75:15) at level ($P < 0.05$). While fiber and ash content were found to be increased but not significantly (Table 5).

3.4. Effect of adding SDYP on the baking attributes of functional cookies

Descriptive analysis of the physical profile of different treatments was conducted. Regarding the diameter attribute results concerned, on average, in T0 (44.15 ± 0.01) mm, T1 (44.17 ± 0.01) mm, T2 (44.24 ± 0.01) mm, and T3 (43.37 ± 0.03) mm was investigated. The maximum diameter value (44.25) mm was calculated in T2 sample having 10% SDYP, whereas the least diameter value was examined in T3 sample (43.35) mm containing 15% SDYP. Thickness in T0 (1.50 ± 0.01) mm, T1 (1.52 ± 0.01) mm, T2 (1.57 ± 0.01) mm, and T3 (1.64 ± 0.01) mm was investigated. Whereas the maximum diameter value (9.27) mm was calculated in T3 sample having 15% SDYP whereas least thickness level was examined in T0 (control) sample (1.49) mm containing 0% SDYP. Regarding the spread ratio attribute results are concerned, on average T0 (29.80 ± 0.03), T1 (30.20 ± 0.01), T2 (30.77 ± 0.02) and T3 (31.09 ± 0.02) was investigated. The maximum spread ratio value (31.10) was calculated in T3 sample having 15% SDYP, whereas the least spread ratio was measured in T0 sample (29.78) containing 0% SDYP. Regarding the color attribute results concerned, on average T0 (156.86 ± 0.55), T1 (165.16 ± 1.10), T2 (173.08 ± 0.44) and T3 (180.97 ± 0.06) were investigated. The maximum color (181.01) was observed in T3 sample having 15% SDYP. In contrast, least color value was examined in T0 sample (156.47) containing absolutely 0% SDYP, as shown in Table 6 and Figure 3.

3.5. Effect of adding SDYP on bioactive profile of functional cookies

As shown in Table 7, descriptive analysis of DPPH inhibition in different Treatments were analyzed. As far the results are concerned, on average in T0 (61.30 ± 0.01) AAE mg/100 g, T1 (67.18 ± 0.01) AAE mg/100 g, T2 (69.93 ± 0.28) AAE mg/100 g and in T3 (72.80 ± 0.16) AAE mg/100 g was observed. The maximum DPPH inhibition (72.91) AAE mg/100 g, also known as antioxidant activity, was found in T3 sample having 15% SDYP whereas minimum DPPH inhibition was calculated in control (61.29) AAE mg/100 g samples developed from absolutely no SDYP. Descriptive analysis of ABTS inhibition in different treatments were analyzed. As far the results are concerned, on average in T0 (37.34 ± 0.21) AAE mg/100 g, T1 (50.26 ± 0.19) AAE mg/100 g, T2 (59.67 ± 0.49) AAE mg/100 g, and in T3 (78.14 ± 0.33) AAE mg/100 g was observed. The maximum ABTS inhibition (78.37) AAE mg/100 g also known as antioxidant activity was found in T3 sample having 15% SDYP whereas, minimum ABTS inhibition was calculated in control (37.19) AAE mg/100 g samples developed from absolutely no SDYP. Descriptive analysis of TPC in different treatments was analyzed. As far as the results are concerned, on average, in T0 (4.95 ± 0.02) GAE mg/100 g, T1 (7.30 ± 0.07) GAE mg/100 g, T2 (10.22 ± 0.13) GAE mg/100 g, and in T3 (16.18 ± 0.28) GAE mg/100 g was observed. The maximum TPC content (16.37) GAE mg/100 g, also known as phenolics, was found in the T3 sample having 15% SDYP. In contrast, the minimum TPC was calculated in control (4.93) GAE mg/100 g samples developed from absolutely no SDYP. In this current study, by partially adding SDYP in the development of functional bread; overall ABTS, DPPH inhibition as well as TPC content was found significant increase in the following order (Wheat: SDYP) of T0 (100:0) < *T1* (95:05) < *T2* (90:10) < *T3* (85:15) at level ($P < 0.05$; Figure 4).

3.6. Effect of adding SDYP on the sensory profile of functional cookies

On average, scores for appearance profile examination depicts that the maximum (8.8) score for color was observed in T3

(15% SDYP) samples as far least (8.2) score was calculated in T0 (control) samples. Although, flavor profile examination depicts that the maximum (8.4) score for flavor was observed in T2 (10% SDYP) samples as far least (7.6) score was calculated in T3 (15% SDYP) samples. Moreover, the taste profile investigation depicts that the maximum (8.3) score for taste was defined in T2 (10% SDYP) samples as far least (7.8) score was calculated in T3 (15% SDYP) samples. On average, scores for color in T0 (8.3), T1 (8.7), T2 (8.8), and T3 (8.9) were detected. Although, color profile examination depicts that the maximum (8.9) score for color was defined in T3 (15% SDYP) samples as far least (8.3) score was measured in T0 (0% SDYP) samples. By adding SDYP concentrations in the development of cookies, the overall acceptability was increased gradually (Figure 5). Meanwhile, results from T2 having 10% SDYP levels, respectively exhibits that SDYP can be added in certain amounts as discussed earlier to fulfill the nutritional gap generated by typical wheat cookies primarily prepared with wheat flour while without losing any of its sensory attributes at level ($P < 0.05$; Table 8).

4. Discussion

During the spray drying of yogurt, it was observed that by increasing the outlet and inlet temperatures while keeping feed temperature constant, protein content and lactose content in dried yogurt powder tend to increase significantly. In contrast, a reduction in moisture content was investigated. In addition, there was no significant effect on pH readings examined. These results were found in engagement with the outcomes reported by Bielecka and Majkowska (18), Fang et al. (46), Guerin et al. (47), and Lam and Nickerson (48). Protein is a heat-sensitive counterpart of a food system; meanwhile, particularly in milk, it becomes more apparent; Lam et al. (48) explained that increasing protein and lactose at constant feeding is mainly due to the rapid evaporation coupled with less contact time with heat source. Effect of drying on the survival of inoculated culture was also examined, which shows that by increasing the outlet and inlet temperatures while keeping feed temperature constant, culture survival in dried yogurt powder tends to decrease significantly, as stated by Barbosa et al. (49).

In contrast, culture B (*L. delbrueckii* subsp. *bulgaricus*) was found significantly affected by the heat treatments whereas, culture A (*S. thermophilus*) was found to resist the heat to an extent. Low temperature (60°C) had a negative effect on powder texture (excessive moisture causing powder lumping). Considering sensory properties, moisture content, and viability of yogurt culture, it was concluded that the outlet air temperature of 70–75°C is optimal for spray drying yogurt. The effect of moisture content of yogurt powder on shelf life during storage will be the subject of further research. Further, a study conducted by Bielecka and Majkowska (18), Rolfe and Daryaei (50), and Khalid et al. (51) explaind that several factors such as the phase of bacterial growth and water activity of dried material along with potential acidity can affect the survival of thermophilic lactic acid bacteria. These results were relevant to the outcomes reported by Bielecka and Majkowska (18), Fang et al. (46), Guerin et al. (47), and Lam and Nickerson (48). A directly proportional relation was investigated between the increasing concentration of SDYP and baking characteristics of cookies (52). Meanwhile, by incorporating SDYP bioactive parameters such as DPPH, the ABTS and TPC count increased significantly (5). The sensory profile shows an incline toward T0 (0% SDYP) to T3 (10% SDYP) in all attributes, i.e., color, flavors, taste, and overall acceptability. Still, it starts to decline when the concentration of SDYP reaches 15%. An inversely proportional trend was observed that increased SDYP concentrations in functional cookies; baking characteristics tended to increase significantly (53). However, these means were studied at confidence level of 95%. In addition, by partially adding SDYP in the development of functional cookies; overall TPC, DPPH and ABTS was found significantly increased in following order of (Wheat: SDYP) of T0 (100:0) < *T1* (95:05) < *T2* (90:10) < *T3* (85:15) at level ($P < 0.05$). It was observed that by adding SDYP concentrations in the development of cookies, the acceptability found to be increased gradually. Meanwhile, results from T2 having 10% SDYP powder levels, respectively exhibits that SDYP can be added in certain amounts as discussed earlier to fulfill the nutritional gap generated by typical wheat cookies primarily prepared with wheat flour while without losing any of its sensory attributes at level ($P < 0.05$).

5. Conclusion

Spray drying is a common method used in the food processing industry to acquire distinctive properties, such as instant solubility and to improve product shelf stability. It is considered the foremost drying approach of the technology used in dairy products due to its low operating costs and high production rates. In this study, yogurt was spray dried using several optimum OAT (65, 70, and 75°C) and IAT (150, 155, and 160°C) settings. Spray drying indicates unequivocally that nutritional loss tends to increase as temperature rises. *Streptococcus thermophilus* culture, on the other hand, exhibits resilience to intense heat approaches, while *L. delbrueckii* subsp. *bulgaricus* culture was found to be considerably impacted. A total of four treatments, including one control, were used to create functioning cookies. The relationship between rising SDYP content and baking properties and cookies' mineral and protein profile was directly proportional. Bioactive measures like DPPH, ABTS, and TPC count were also considerably impacted. The sensory profile shows an incline toward T0 (0% SDYP) to T3 (10% SDYP) in all attributes but starts to decline when the concentration of SDYP reaches 15%. The practical application of this study suggests that by employing a certain combination of temperatures (OAT: 60°C) and (IAT: 150°C), the maximum survival of inoculated culture can be achieved and this powder can be utilized in the development of functional cookies with enhanced sensory as well as biochemical characteristics.

Data availability statement

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

Author contributions

AA: conceptualization. AA, MTJ, DT, and AK: writing-original draft preparation. MTJ, DT, TM, TAD, RS, WK, and TT: writing-review and editing. W-FL: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer AAK declared a shared affiliation with the author WK to the handling editor at the time of review.

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Formulation of fortified instant weaning food from *Musa paradisiaca* (banana) and *Eleusine coracana*

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Weaning food is a soft, easily digestible type of food other than breast milk for infants aged 6 to 24 months. The present study was conducted to develop cereal-fruit-based complementary foods for infants and evaluate the nutritional quality of such types of foods. Few researchers have focused on formulating weaning foods from locally available, nutritious, and rich ingredients without nutrient loss to reduce malnutrition and infant morbidity rates. In this study, the formulated infant food was prepared from *Musa paradisiaca* (*Nendran* banana) and *Eleusine coracana (ragi*). Formulated weaning food was analyzed using various standard methods, demonstrating that it could provide adequate nutrients to growing infants for their proper growth and development. The shelf life of the weaning food was also studied for a period of 3 months at ambient conditions in two different packaging materials: aluminum and plastic (low-density polyethylene or LDPE), with the aluminum foil pouch exhibiting the best shelf life. This ready-to-serve food, which is formulated and fortified with natural ingredients containing essential macronutrients and micronutrients, could be regarded as highly effective supplementary food for infants. Furthermore, this development has the potential to introduce an affordable weaning product specifically targeted at low socioeconomic groups.

KEYWORDS

banana, finger millet, fortification, infants, micronutrients, weaning food

1. Introduction

Weaning food refers to a soft and easily digestible source of nutrition aside from breast milk, which is suitable for infants aged 6 to 24 months. It plays a crucial role in providing necessary nourishment for optimal growth and development in infants. An adequate amount of nutrients play a vital role during an infant's maturity phase. The recommended dietary allowance (RDA) establishes the essential nutrient limits that are essential for the growth and development of infants throughout their 1st year of life. As infants grow, they develop the ability to chew and gradually receive a wide variety of complementary foods into their diet,

expanding beyond solely consuming liquid nourishment such as breast milk (1). Weaning foods could be comparable to breast milk in terms of ease of digestion. However, breast milk lacks many essential nutrients such as vitamin D, iron, and so on Parvin et al. (2). According to the World Health Organization (WHO), infants should be exclusively breastfed for at least the first 6 months, followed by the introduction of nutritious and complementary foods to achieve healthy, optimal feeding (3). Introducing a combination of cereals, pulses, nuts, fruits, and vegetables during the weaning process can provide infants with a well-rounded diet with adequate calories and nutrients. The RDA also outlines the energy requirements for individuals. The WHO recommends the following RDAs: carbohydrates, 50–95 g; protein, 9.1–11 g; fat, 30–31 g; fiber, 1–4.4 g; vitamin B1, 0.1–0.2 mg; vitamin B2, 0.3–0.4 mg; vitamin B3, 2–4 mg; vitamin B6, 0.1–0.3 mg; vitamin C, 40–50 mg; calcium, 210–270 mg; sodium, 100–200 mg; potassium, 200–700 mg; and iron, 0.27–10 mg (1).

Fruits are an important constituent of the human diet, as they are one of the most important sources of vitamins and minerals. *Musa spp.*, comprising banana and plantain, are among the world's leading fruit crops. Worldwide, 125 million tons were produced in 2021, according to the Food and Agriculture Organization (FAO) statistics database (4). Bananas are easily available fruits that are rich in fiber, vitamins, and minerals, which offer great health benefits, especially for babies, making them an excellent supplementary food option. India ranks first among producers in the cultivation of bananas (5). Among the wide varieties of this fruit, Nendran *(Musa paradisiaca)* is considered a supreme variety for its carbohydrate and micronutrient content (6). Nendran banana is typically preferred as a complementary food option since it helps with weight gain and provides the necessary nutrients for babies' growth and development. They are well-known for their texture and aroma and are a good source of potassium, sodium, and calcium (7). Boiled and mashed bananas are an excellent nutrient source for infants, even from 4 months onward. In many countries, mothers choose bananas as the first solid food to introduce to their babies when beginning their solid food intake (8). The soft, mushy texture of bananas helps facilitate easier digestion for babies.

Finger millet (*Eleusine coracana* L.), also known as African millet, is commonly called *ragi* in India. It has excellent nutritional value and functional properties and is superior to other common cereals (9). In other parts of the world, the first food to be introduced to infants is traditionally cereal. Cereals are a major source for the development of weaning food. *Ragi's* mineral content is high when compared to rice and wheat. It is rich in both calcium and iron. No other cereal comes close to *ragi* regarding its calcium content (10). Calcium is an essential micronutrient for children's bone growth and development. *Ragi* can potentially replace calcium pills.

Additionally, it contains B vitamins such as thiamine, riboflavin, and niacin. It is a good source of carbohydrates, protein, and dietary fiber and has a lower natural fat content than other cereals. The fat in *ragi* is unsaturated; hence, it can be used as a substitute for rice and wheat to reduce weight gain (10). All the essential nutrients in *ragi* make it suitable for large-scale production and the manufacturing of different food products. Thus, *ragi* can be used to prepare ready-to-eat or ready-to-cook products to enhance their consumption (11). Therefore, *ragi* is considered one of the most important staple foods for low-income people (11).

Preserving food nutrients with an extended shelf life is challenging for researchers, particularly micronutrients such as vitamins and minerals in bananas and *ragi*. Different drying techniques should be explored to preserve the nutrient content of raw materials. In this study, we used the freeze-drying technique to obtain banana and *ragi* powder to develop weaning food. Freeze drying has been considered the most advanced method of drying, as it helps retain micronutrients without causing any damage to micronutrients and preserves food quality. Therefore, heat-sensitive products can be dried using this method without changing nutritional quality, taste, aroma, flavor, or color. Fortification is the practice of improving the nutritional quality of food products and increasing the content of essential micronutrients to provide high-quality products with minimal risks. Food fortification has been enacted as a public health policy in many countries. Fortification is mainly carried out to ensure that minimum dietary requirements are met. Fortified food reduces the risk of malnutrition in infants, along with breast milk intake. Fortification is an effective way to prevent micronutrient deficiencies. Severe forms of malnutrition change the infant's body structure, physiology, and metabolism.

Consequently, it is essential to add carbohydrates and protein-rich ingredients such as bananas and *ragi* to the weaning food formulation (8). At 6 months of age and beyond, most infants begin to eat supplementary semisolid food. At this stage, fortified weaning food is said to play a major role in their nutrition (12, 13).

In short, weaning food is crucial for the development of infants' physical, brain, and immune systems. Bananas and *ragi* are rich in minerals such as calcium, sodium, potassium, iron, and vitamins such as B1, B2, B3, B6, and C. During the processing of bananas and *ragi*, there is a possibility for a decrease in the number of micronutrients, which is compensated for through fortification in infant food. In addition, bananas and *ragi* lack certain essential vitamins, such as vitamin A, vitamin E, and vitamin B12, that are essential for the growth and development of infants. Therefore, the addition of a premix containing these deficient vitamins per the RDA through fortification was carried out in this research (14).

The aim of this study was to develop nutritionally fortified weaning food from dried banana and *ragi* per RDA and to evaluate the quality of dried weaning food at atmospheric conditions using aluminum and low-density polyethylene (LDPE) pouches as packing materials.

2. Materials and methods

2.1. Sample preparation and weaning food development

Banana (7th stage), *ragi* flour, skimmed milk powder, whey protein, and dates powder were collected from the local market in Potheri, Chennai, Tamil Nadu, India. The edges of the banana were trimmed and cut into two pieces from the center. It was then steam-cooked at 100°C ± 3°C by indirect steaming for 10 min over medium heat. The bananas were peeled off and slit to remove the seeds from the center. Then, the pieces were transferred into a

mixer jar and pulsed once. Afterward, water was added to obtain the desired consistency. The obtained puree was dried using the freeze-drying technique. The steam-cooked banana pulp was pre-frozen by spreading as a 3-mm layer inside a deep freezer at −18°C for 2 days. The pre-frozen sample was placed inside a freeze drier and then automatically dried under vacuum (10 Pa absolute pressure) in the freeze drier (Lyodel,153-06-10, India) at −45°C for 16 h. *Ragi* flour was boiled similarly on medium heat until a porridge-like consistency was obtained. Then, the thick porridge was transferred onto three plates and spread uniformly for freeze-drying per the above protocol. A study conducted by Kabeer et al. (5) indicated that drying can increase the quality and shelf life of bananas. Therefore, following the RDA, both dried powders and other ingredients, such as skimmed milk powder, whey protein, and dates powder, were thoroughly mixed in three different ratios based on their major nutrient content. Subsequently, the most optimal formulation was chosen based on proximate composition and micronutrient analyses.

2.2. Proximate analysis

Proximate analysis was performed on the formulated weaning foods. Moisture content was determined by drying the samples at 104°C in a hot air oven until a constant weight was attained (15). Carbohydrate content was determined using the anthrone method (16). Moreover, acid hydrolysis was carried out to break down polysaccharides into simple sugars. The resultant monosaccharides were estimated using a spectrophotometer at a wavelength of 670 nm. Protein content was determined using the Kjeldahl method (17). This method determines the nitrogen content of the sample. The amount of protein was calculated by multiplying nitrogen content with a 6.25 conversion factor. The fat content of the samples was determined using the Soxhlet extraction method (18). Fat extraction was conducted using hexane as the solvent. After 6 h, fat content was measured. The dietary fiber of the samples was determined using acid-alkali hydrolysis (19). Ash content was determined using a muffle furnace. After removing moisture content, samples were kept in the muffle furnace at 500°C for 3 h (20). Each analysis was conducted in triplicate, and values were expressed on a dry-weight basis.

2.3. Micronutrient analysis

2.3.1. Determination of minerals

Mineral contents from the formulated weaning food were analyzed with the use of a flame emission atomic absorption spectrophotometer (AAS) at a wavelength of 598 nm. Moreover, 10 g of each formulated sample was placed in a porcelain dish. Then, it was placed inside a muffle furnace at 500°C for 5 h. The samples were transferred into a 250-ml beaker and mixed with 50 ml of deionized water and 15 ml of concentrated nitric acid. The beaker was then heated over a hot plate. Then, the samples were filtered and brought to a final volume of 250 ml by adding deionized water. The standard stock solution of 100 ppm was prepared. The atomic absorption instrument was set up. Then, samples, along with blanks and standards, were read at 589 nm using the AAS. The mineral content was statistically analyzed by developing a calibration curve. The analysis was conducted in triplicate, and values were expressed on a dry-weight basis.

2.3.2. Determination of vitamins

The formulated weaning food contained vitamins such as B1, B2, B3, B6, and C; this vitamin content was analyzed through high-performance liquid chromatography (HPLC). Standards for vitamins B1, B2, B6, and C were prepared by accurately weighing 10–20 g of vitamin powder in 1 ml of deionized water, while for vitamin B3, the standard was prepared by adding 10–20 g of vitamin powder to 0.5 ml of potassium hydrogen carbonate. Working samples were prepared by accurately weighing 0.100 g of dried powder, adding 80 ml of water, and mixing properly. Then, the samples were centrifuged at 4,000 rpm for 25 min. Afterward, the supernatant was collected for vitamin analysis along with the standard. The sample solution was filtered through a 0.25-μm filter. The analysis was conducted using Acclaim PA columns with dimensions of 3.0 × 150 mm and a particle size of 3 μm. The column temperature was maintained at 25°C. The mobile phases were a 25-mm phosphate buffer (pH 3.6) and acetonitrile, with a flow rate of 0.5 mL/min. The vitamin content was calculated based on the methods described in other studies (21), with the aid of retention time and peak area. The analysis was conducted in triplicate, and the results were expressed on a dry-weight basis.

2.4. Fortification of weaning food

During the processing of bananas and *ragi*, there may be a high probability of losing some vitamins and minerals. Fortification is the process of replenishing lost micronutrients. A recent study (22) has revealed that fortification has a positive impact on the growth of infants. Weaning food contains vitamins such as vitamins B1, B2, B3, B6, and C and minerals (such as sodium, potassium, calcium, and iron), and any loss in these micronutrients in food can be added externally via fortification. Some vitamins (such as A, E, and B12) are necessary for the growth of infants but are absent in bananas and *ragi*. Thus, they must be added externally as fortifications to meet the RDA. Food-grade vitamins and minerals were obtained from Hexagon Nutrition (Exports) Private Limited in Chennai, Tamil Nadu, India, and used for fortifying the formula.

2.5. Physiochemical analysis

Various important physical properties such as water activity, water holding capacity, viscosity, bulk density, and the pH of the selected weaning food were determined. Water activity was measured using a water activity meter (Novasina LabSwift-AW, India) available at the Department of Food and Process Engineering, SRMIST. The water holding capacity was determined by placing 1 g of dried sample into a centrifuge tube containing 10 ml of distilled water with proper mixing. It was then centrifuged at 5,000 rpm for 25 min. The supernatant was discarded, and the residue was weighed with the centrifuge tube. The weight difference provided the value of the water's holding capacity.

Viscosity was determined by mixing 3 g of weaning food with 27 ml of distilled water at 30°C. This slurry was used for determining viscosity. Viscosity was measured in Brook's field viscometer using spindle number 18, rotating at 100 rpm. Bulk density was represented as the ratio of the mass of the sample to the volume of the sample (23). The pH was determined by putting 1 g of the sample in a centrifuge tube containing 10 ml of distilled water and mixing it appropriately. It was then centrifuged at 3,000 rpm for 15 min. The supernatant was collected for pH determination and measured using Systronics digital pH meter 335 (24).

2.6. Shelf-life analysis

The shelf life of weaning food was studied for 3 months at 32° ± 3°C by packing the product in two different packaging materials: aluminum and plastic (low-density polyethylene, LDPE). Proximate, micronutrient, physiochemical, and microbial analyses were also part of the shelf-life study. Proximate micronutrient and physiochemical measurements were conducted in accordance with the above procedures. Microbial analysis of the weaning food was performed to assess the bacterial, fungal, and yeast load under laboratory conditions. All media and equipment were sterilized under steam sterilization at 15 psi for 20 min at 121°C in an autoclave. For analysis, 1 g of each sample was weighed and diluted to 1:10 (1 g in 9 ml of water) with distilled water. A serial dilution was prepared and the spread plate technique was performed. Total plate count (TPC) was determined using a nutrient agar (NA) plate, and for yeast and mold, potato dextrose agar (PDA) plates were used. Samples were spread on the agar plates with the help of glass rods (25, 26). The plates were then incubated at 37°C for 24 to 72 h.

2.7. Statistical analyses

The data were analyzed statistically using a one-way ANOVA. All the analysis was conducted in triplicate, and the data were expressed as mean ± SD, and significance was accepted at a p-value of < 0.05 per Duncan's multiple range test.

3. Results

3.1. Development of weaning food

For product development, three different formulations were created from the freeze-dried banana and *ragi* powders (Table 1). Formulations were created based on the RDA requirements. Besides bananas and *ragi*, raw materials remained constant in these three formulations. The best formulation was selected based on the proximate and micronutrient analyses. FB-Formulation 2 fulfilled the necessary RDA criteria.

TABLE 1 Different formulations for preparing weaning foods.

Ingredients	Formulation-I (g/100 g)	Formulation-II (g/100 g)	Formulation-III (g/100 g)
Banana powder	45	40	50
Ragi powder	35	40	30
Skimmed milk powder	10	10	10
Whey protein	5	5	5
Dates powder	5	5	5

3.2. Proximate analysis of weaning food

The results of proximate analysis for different formulations are described graphically in Figure 1. The results showed that the second formulation was the best, as the values of the proximate components of FB-Formulation 2 were very close to those of the RDA. The RDA for the carbohydrate content for infants is between 50 and 95 g/100 g. The carbohydrate content of the formulated weaning food was determined to be 92 g/100 g. The protein content of the formulated weaning food was found to meet the RDA. The RDA for the protein content for infants was estimated to be 9.20 g/100 g. Ash content corresponded to its mineral content. The formulated weaning food had a high amount of ash content (4.57 g/100 g). The data were analyzed statistically using one-way ANOVA via Minitab. All the analyses were conducted in triplicate, and the data were expressed as mean ± SD, and significance was accepted at a p-value of < 0.05 per Duncan's multiple tests.

3.3. Micronutrient analysis of weaning food

Figures 2A, B describes the vitamin and mineral analyses for different formulations of weaning foods. The results indicated that all other micronutrients met the infant's RDA requirements except sodium and vitamin C contents. The results also showed that FB-Formulation 2 was best for providing a weaning infant with adequate micronutrients based on the infant's RDA. The data were analyzed statistically using a one-way ANOVA via Minitab. All the analyses were conducted in triplicate, and the data were as expressed as mean ± SD, and significance was accepted at a p-value of < 0.05 per Duncan's multiple tests.

The RDA of sodium content for weaning food was within the range of 1–2 mg/g. However, the sodium content of the formulated weaning food was 431 μg/g. The RDA of vitamin C content was within the range of 40–50 mg/100 g. However, vitamin C content was 250 μg/g in the formulated freeze-dried weaning foods. The possible reason for these trivial values is that the drying banana procedure to obtain a powder reduces the content of both sodium and vitamin C. Therefore, fortification was carried out to increase the nutrient content of the formulated weaning food.

The results indicated that all the other micronutrients met the infant's required RDA except sodium and vitamin C content. The results also confirmed that FB-Formulation 2 was best for developing weaning foods with an adequate number of micronutrients.

3.4. Fortification of weaning food

FB-Formulation 2 was selected for the development of fortified weaning foods based on the results of proximate

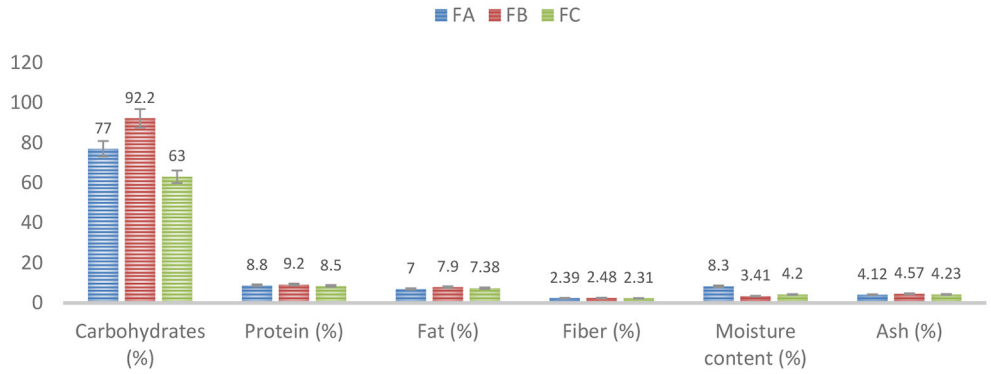

FIGURE 1
Proximate analysis for different formulations, FA-Formulation 1, FB-Formulation 2, and FC- Formulation.

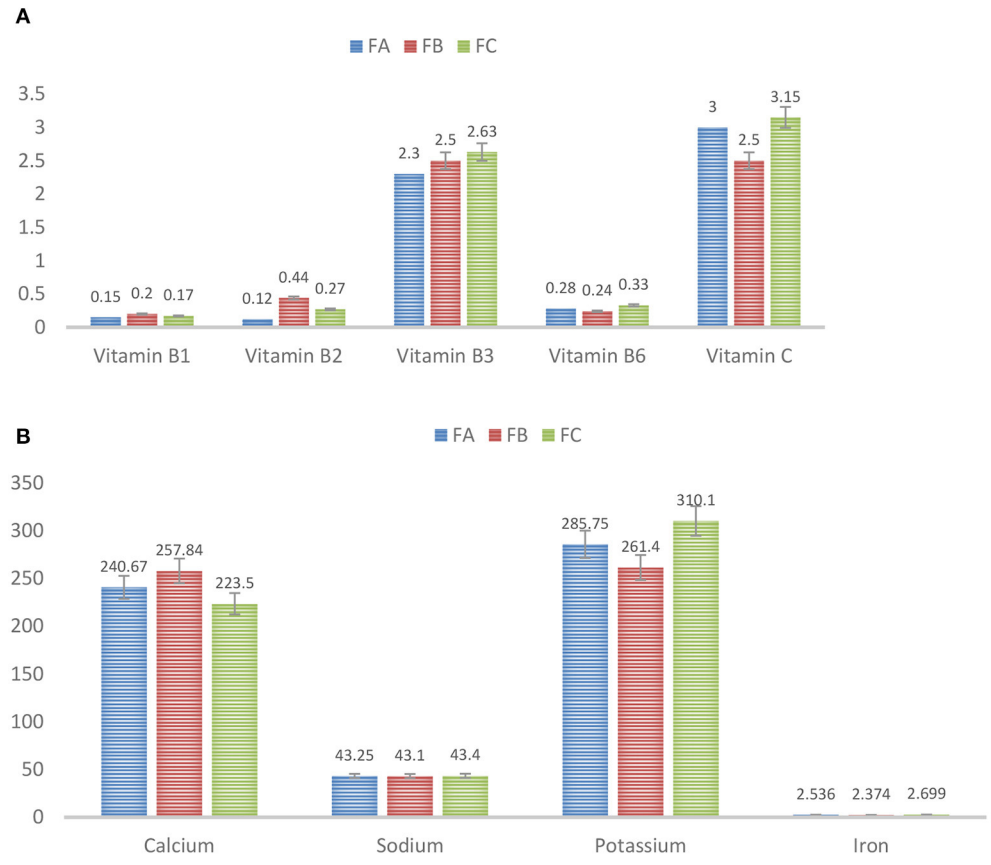

FIGURE 2
(A) Vitamin analysis for different formulations FA-Formulation 1, FB-Formulation 2, and FC- Formulation 3. **(B)** Mineral analysis for different formulations FA-Formulation 1, FB-Formulation 2, and FC- Formulation 3.

and micronutrient analysis. This formulation contained an equal amount of *ragi*, banana powder, and other ingredients, as mentioned above (Table 1). Owing to certain nutrient insufficiencies, fortification with a premix containing these deficient vitamins as per the RDA was employed.

3.5. Shelf-life analysis

The shelf life of weaning food was studied for 3 months by packing the product in two different packaging materials: aluminum and LDPE. Changes in the functional properties and micronutrient content during the shelf-life study are depicted

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TABLE 2 Changes in the functional properties of freeze-dried weaning food during its shelf life.

Functional properties	Day 0	Day 15		Day 30		Day 45		Day 60		Day 75		Day 90	
		Al	LDPE	Al	LDPE	Al	LDPE	Al	LDPE	Al	LDPE	Al	LDPE
Carbohydrates (%)	91 ± 0.23^{a}	91 ± 0.04^{a}	91 ± 0.02^{a}	90 ± 0.02^{a}	90 ± 0.05^{b}	$90. \pm 0.02^{b}$	89 ± 0.01^{b}	90 ± 0.01^{b}	$89. \pm 0.04^{b}$	90 ± 0.01^{c}	88 ± 0.04^{c}	89 ± 0.01^{c}	88 ± 0.01^{c}
Fat	7.9 ± 0^{a}	6.8 ± 0.46^{b}	6.8 ± 0.07^{b}	6.6 ± 0.02^{c}	6.5 ± 0.02^{c}	6.3 ± 0.03^{c}	6.3 ± 0.01^{c}	6.1 ± 0.05^{c}	6.1 ± 0.01^{c}	6.1 ± 0.04^{c}	5.9 ± 0.01^{c}	6.0 ± 0.05^{c}	5.9 ± 0.05^{c}
Fiber (%)	2.4 ± 004^{a}	2.4 ± 0^{a}	2.4 ± 0.1^{a}	2.4 ± 0.07^{a}	2.3 ± 0.08^{b}	2.3 ± 0.08^{b}	2.3 ± 0.03^{b}	2.3 ± 0.1^{b}	2.3 ± 0.2^{b}	2.3 ± 0.4^{b}	2.3 ± 0.1^{b}	2.3 ± 0.1^{b}	2.2 ± 0.1^{b}
Ash content (%)	4.5 ± 0.04^{a}	4.4 ± 0.35^{a}	4.4 ± 0.21^{a}	4.4 ± 0.08^{b}	4.3 ± 0.1^{b}	4.3 ± 0.2^{b}	4.2 ± 0.07^{c}	4.3 ± 0.47	4.2 ± 002^{c}	4.2 ± 0.01^{c}	4.1 ± 0.041^{c}	4.2 ± 0.02^{c}	4.16 ± 0.03^{c}
Moisture content (%)	3.4 ± 0.21^{a}	3.4 ± 0.012^{a}	3.4 ± 0.01^{a}	3.9 ± 0.05^{b}	4.21 ± 0.01^{b}	4.53 ± 0.04^{b}	4.98 ± 0.07^{b}	4.78 ± 004^{b}	5.34 ± 0.1^{c}	4.8 ± 0.02^{b}	5.38 ± 0.12^{b}	4.82 ± 0.5^{c}	5.4 ± 0.31^{c}
Bulk density (g/cm^3)	0.56 ± 0.4^{a}	0.404 ± 24^{a}	0.42 ± 23^{a}	0.40 ± 0.24^{a}	0.42 ± 0.4^{a}	0.40 ± 0.21^{a}	0.424 ± 21^{a}	0.404 ± 13^{a}	0.42 ± 0.1^{a}	0.35 ± 0.1^{b}	0.424 ± 24^{a}	0.35 ± 0.4^{b}	0.424 ± 0.5^{a}
Water holding capacity	9.62 ± 0.36^{a}	9.62 ± 37^{a}	9.29 ± 14^{a}	$9.54 \pm 0.6a$	9.21 ± 14^{a}	9.52 ± 0.45^{a}	9.14 ± 24^{a}	9.47 ± 32^{a}	9.12 ± 0.1^{a}	9.39 ± 23^{a}	8.25 ± 13^{b}	9.39 ± 0.8^{a}	8.25 ± 0.4^{b}
Water activity (aw)	0.42 ± 0.35^{a}	0.42 ± 0.5^{a}	0.42 ± 0.28^{a}	0.48 ± 0.9^{a}	0.494 ± 32^{a}	0.45 ± 0.9^{a}	0.478 ± 31^{a}	0.50 ± 0.1^{a}	0.52 ± 0.1^{b}	0.504 ± 17^{b}	0.543 ± 10^{b}	0.50 ± 0.6^{a}	0.51 ± 0.2^{a}
Viscosity (cP)	1.05 ± 0.4^{a}	1.5 ± 0.7^{b}	1.56 ± 0.6^{b}	1.52 ± 0.4^{b}	1.62 ± 0.4^{b}	1.86 ± 0.4^{b}	2.31 ± 2.1^{c}	2.38 ± 0.4^{c}	2.6 ± 0.31^{c}	2.52 ± 0.9^{c}	2.86 ± 0.1^{c}	2.13 ± 0.1^{c}	2.54 ± 0.8^{c}
pH	6.19 ± 0.2^{a}	6.19 ± 0.4^{a}	6.29 ± 0.4^{a}	6.34 ± 0.2^{a}	6.4 ± 1.8^{a}	6.36 ± 0.4^{a}	6.46 ± 1.3^{b}	6.32 ± 0.6^{a}	6.46 ± 2.3^{b}	6.36 ± 1.6^{a}	6.48 ± 0.7^{b}	6.36 ± 0.3^{a}	6.49 ± 0.4^{b}

Each data point represents the mean ± standard deviation of triplicate analysis. The multiple comparisons were determined using Duncan at a p-value of <0.05 columns with significantly different letters ($p < 0.05$).

in Tables 2, 3. The results indicate that changes are detected in carbohydrates, fat, moisture content, water activity, viscosity, and pH values of weaning food packed in different packaging materials. The data were analyzed statistically by one-way ANOVA using Minitab. The significant difference increases when the storage life increases. Statistical analysis revealed that products packed in plastic packaging show a significantly higher increase when compared to products packaged in aluminum. Microbial growth during storage is presented graphically and expressed as log CFU/ml. Figure 3 shows the bacterial and fungal growth of weaning food during its shelf life. The microbial load appeared to be under control upon extended storage and can be considered safe for infants' consumption.

3.6. Figures and tables

The series of figures and tables presented below describe our research findings and are referenced in the text in the Results section.

4. Discussion

An infant's firs1stt year is crucial for its growth and development. Typically, in the first 6 months, growth and weight gain are pronounced, while in the second half of the first year, they are not as rapid. Infants experience a wide range of developmental milestones in interacting, learning, speaking, behaving, and movement (1–3). Adequate nutrition is vital for brain development, especially during pregnancy and infancy. The introduction of complementary feeding, i.e., semisolid weaning food around 6 months of age and breast milk feeding or infant formula, is encouraged and highly recommended. In low-income countries (LICs) or lower-middle-income countries (LMICs), as well as low socioeconomic groups, good-quality weaning foods or practices are a tremendous challenge. This presents a real dilemma for mothers regarding infant feeding and maintaining a suitable nutritional status. Locally sourced ingredients potentially mitigate nutritional risks (4–8).

For example, India has become the world's most populous country. The 2021 gross national income (GNI) per capita is between $1,086 and $4,255. After mangoes, banana variety production is India's most important fruit crop. On average, the output is ~29 million tons per year, mostly serving the domestic market (FAO website) (4, 5). Considering its market availability and nutritional composition, bananas offer an opportunity to develop affordable, appropriately formulated, fortified weaning food for infants for local market consumption.

The process of formulating weaning food involves two crucial components: product development, quality control, and regulatory approval. First, it entails determining the nutrient content of the formulation, which includes analyzing the proximate composition, and micronutrient constituents and implementing fortification. Second, it involves assessing and ensuring the shelf life of the developed product.

TABLE 3 Changes in the micronutrient content of freeze-dried weaning food during its shelf life.

Micronutrients (mg/100 g)	Day 0	Day 15		Day 30		Day 45		Day 60		Day 75		Day 90	
		Al	LDPE	Al	LDPE	Al	LDPE	Al	LDPE	Al	LDPE	Al	LDPE
Vitamin B1	0.2 ± 0.04^{a}	0.2 ± 0.01^{a}	0.2 ± 0.1^{a}	0.2 ± 0.2^{a}	0.2 ± 0.1^{b}	0.2 ± 0.2^{b}	0.1 ± 0.1^{c}	0.1 ± 0.1^{c}	0.1 ± 0.4^{c}	0.1 ± 0.1^{c}	0.1 ± 0.5^{c}	0.1 ± 0.2^{c}	0.1 ± 0.1^{c}
Vitamin B2	0.5 ± 0.03a	0.5 ± 0.03^{a}	0.48 ± 0.3^{b}	0.517 ± 0.3^{a}	0.41 ± 0.3^{b}	0.4 ± 0.01^{b}	0.3 ± 0.2^{c}	0.4 ± 0.2^{b}	0.3 ± 0.1^{c}	0.4 ± 0.1^{b}	0.3 ± 0.7^{c}	0.41 ± 0.3^{b}	0.31 ± 0.6^{c}
Vitamin B3	2.5 ± 0.12^{a}	2.45 ± 0^{a}	2.43 ± 0.5^{a}	2.42 ± 0.1^{a}	2.37 ± 0^{b}	2.35 ± 0.1^{a}	2.3 ± 0.04^{b}	2.34 ± 2^{a}	2.26 ± 3^{b}	2.31 ± 2^{b}	2.22 ± 7^{b}	2.29 ± 1^{b}	2.16 ± 0.1^{c}
Vitamin B6	0.24 ± 0.04^{a}	0.23 ± 0.2^{a}	0.19 ± 0.3^{b}	0.21 ± 0.62^{a}	0.17 ± 0.1^{b}	0.19 ± 0.1^{b}	0.15 ± 0.1^{c}	0.186 ± 0.2^{b}	0.134 ± 0.4^{c}	0.18 ± 0.1b	0.13 ± 0.4^{c}	0.16 ± 0.1^{c}	0.13 ± 0.7^{c}
Vitamin C	43.5 ± 0.05^{a}	43.2 ± 0.1^{a}	43.1 ± 0.1^{a}	43.1 ± 0.1^{a}	43.1 ± 0.1^{a}	43.1 ± 0.2^{a}	43.0 ± 0.1^{a}	43.0 ± 0.1^{a}	42.5 ± 0.1^{b}	42.9 ± 0.6^{b}	42.1 ± 0.3^{b}	42.8 ± 0.1^{c}	42.0 ± 0.2^{c}
Vitamin A	450 ± 0.1^{a}	449.9 ± 0^{a}	449.7 ± 0.3^{a}	449.7 ± 0.4^{a}	449.2 ± 0.7^{a}	448.6 ± 0.3^{b}	448.3 ± 0.2^{b}	447.6 ± 0.4^{c}	447.3 ± 0.7^{c}	446.0 ± 0.1^{c}	445.9 ± 0.7^{c}	445.9 ± 0.3^{c}	445.6 ± 0.7^{c}
Vitamin B12	0.45 ± 0.1^{a}	0.42 ± 0.1^{a}	0.421 ± 0.4^{a}	0.413 ± 0.1^{b}	0.419 ± 0.4^{b}	0.413 ± 0.1^{b}	0.406 ± 0.1b	0.412 ± 0.1^{c}	0.404 ± 0.4^{c}	0.413 ± 0.7^{c}	0.404 ± 0.1^{c}	0.413 ± 0.4^{c}	0.401 ± 0.1^{c}
Vitamin E	4.5 ± 0.1^{a}	4.48 ± 0.1^{a}	4.45 ± 0.3^{a}	4.45 ± 0.1^{a}	4.45 ± 0.1^{a}	4.42 ± 0.1^{b}	4.40 ± 0.1^{b}	4.40 ± 01^{b}	4.40 ± 0.5^{b}	4.395 ± 0.1^{c}	4.33 ± 0.01^{c}	4.37 ± 0.05^{c}	4.31 ± 0.04^{c}
Calcium	257.84 ± 0.01^{a}	257.4 ± 0.1^{a}	257.2 ± 0.1^{a}	257.1 ± 40.3^{a}	257 ± 0.1^{a}	257 ± 0.4^{a}	256.9 ± 0.1^{b}	257 ± 0.2^{a}	256.9 ± 0.5^{b}	256.9 ± 0.9^{b}	256.8 ± 0.1^{b}	256.8 ± 0.2^{c}	256.7 ± 0.1^{c}
Sodium	143.2 ± 0.01^{a}	142.8 ± 0^{a}	142.6 ± 0.9^{a}	142.5 ± 0.1^{a}	141.9 ± 0.5^{b}	142.06 ± 0.8^{a}	141.09 ± 0.1^{b}	141.2 ± 0.1^{b}	140.2 ± 0.4^{c}	140.3 ± 0.1^{b}	139.5 ± 0.7^{c}	140.3 ± 0.1^{b}	139.4 ± 0.6^{c}
Potassium	261.4 ± 0.01^{a}	261.3 ± 0.1^{a}	261.1 ± 0.1^{a}	261.24 ± 0.23^{a}	261.1 ± 0.7^{a}	261.2 ± 0.4^{b}	261.04 ± 0.2^{b}	261.1 ± 0.1^{b}	261 ± 0.21^{b}	261 ± 0.12^{b}	260.9 ± 0.1^{b}	260.9 ± 0.1^{b}	260.8 ± 0.2^{b}
Iron	2.31 ± 0.04a	2.3 ± 0.1^{a}	2.28 ± 0.04^{a}	2.29 ± 0.1^{b}	2.21 ± 0.1^{a}	2.27 ± 0.3^{b}	2.1 ± 0.5^{b}	2.2 ± 2^{b}	2.15 ± 24^{c}	2.2 ± 0.14^{c}	2.11 ± 1^{c}	2.18 ± 123^{c}	2.1 ± 0.24^{c}

Each data point represents the mean ± standard deviation of triplicate analyses. The multiple comparisons were determined using Duncan at a p-value of <0.05 columns, with the different letters being significantly different ($p < 0.05$).

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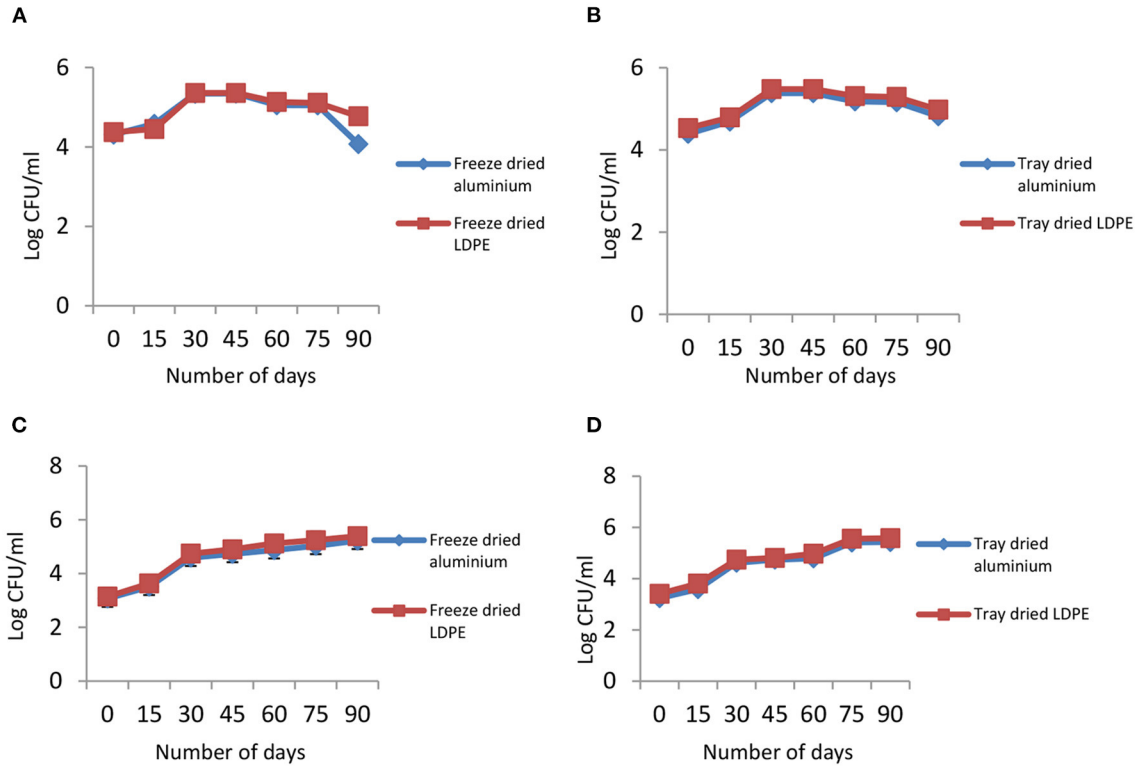

FIGURE 3
Changes in microbial load during the shelf-life of fortified weaning foods. **(A)** Bacterial growth of freeze-dried weaning. **(B)** Bacterial growth of tray-dried weaning food during shelf-life. **(C)** Fungal growth of freeze-dried food. **(D)** Fungal growth of tray-dried weaning food during shelf-life.

Ingredients for the weaning food formulation were sourced locally. Three different formulations were explored: FA-Formulation 1, FB-Formulation 2, and FC-Formulation 3. Sample preparation and mix ratios of constituents are provided in detail above (Table 1) (5). The best formulation was selected based on the proximate composition (a measure of moisture, ash, lipid, protein, and carbohydrate contents) and micronutrient analyses (a measure of vitamins and minerals). Based on the analytical findings, Formulation 2 was deemed best and was selected for further studies [proximate analysis, (Figure 1) and vitamins & minerals analyses, (Figure 2)] utilizing two different packaging materials, aluminum and LDPE (Tables 2, 3), prepared under freeze-dried and tray-dried conditions.

In the following paragraphs, the discussion will focus on two processes involved in the development of weaning foods. In the future, we will ascertain the optimal formulation/fortification and appraise the quality content investigation part before discussing experimental shelf-life interpretation.

4.1. Formulation and content assessment

The freeze-dried FB-Formulation 2 weaning food carbohydrate content was 92 g/100 g, while the tray-dried one was 57 g/100 g (data not shown), which is within the value limits of RDA criteria. The RDA recommendation for carbohydrates for infants is 50–95 g/100 g. The protein content for both methods was found to be nearly identical and meet the RDA requirement. Even though freeze-dried weaning food had a slightly higher protein content (data not shown), the ash content, which reflects the mineral content of the formulation, was somewhat higher in the freeze-dried weaning food compared to the tray-dried weaning food. However, it is important to consider that a high fiber intake may not be advantageous for infants, as their nutritional needs differ in terms of fiber requirements (2). The formulated weaning food had a low fiber content, which, although low, met the suggested RDA value. Comparatively, the weaning formulation contained a lower amount of fat compared to the RDA guidance. However, a food sample with a high fat content increases the risk of spoilage by more than one for a food sample with a lower fat content (27). Hence, a low-fat content reduces the possibility of spoilage and increases shelf life. Moisture content is also an important factor in preserving food products for a longer period (28). FB-Formulation 2 had the least moisture content.

Micronutrient contents met the standard values provided by the RDA except for sodium and vitamin C. The recommended sodium content range is 100–200 mg/100 g. In the formulated freeze- and tray-dried weaning food, sodium content was 43.1 and 42.8 mg/100 g (not shown), respectively. The recommended vitamin C content range is 40–50 mg/100 g. Vitamin C content was 2.5 and 2.1 mg/100 g (not shown) in the formulated freeze- and tray-dried weaning foods. One possible explanation is that freeze-dried bananas reduces the content of both sodium and vitamin C. Generally, freeze-dried powder retains higher amounts of sodium

and vitamin C than tray-dried powder. Therefore, fortification was implemented to increase the content of sodium and vitamin C in formulated weaning foods. Besides, the formulation was enriched with vitamins A, E, and B12, as they are absent in bananas and *ragi*.

4.2. Shelf-life evaluation

Shelf-life experiments on weaning FB-Formulation 2 were conducted on two different packaging materials, aluminum and LDPE, which were prepared either under freeze-dried or tray-dried protocols. Only freeze-dried results are displayed.

The formulated weaning food initially showed the highest bulk density before declining over time. Usually, the higher the bulk density, the lower the moisture content (29). This is a direct indicator of the best storage life for the powder. These results were corroborated by other reports (30). Water holding capacity corresponded with the ability of the powder to absorb water and attain a desired consistency. Formulated weaning food had high water holding capacity, and no significant change was noticed during the storage period, which has also been confirmed previously by other reports (31).

For the freeze-dried concoction, there were no significant changes in the macronutrient (carbohydrate, protein, fat, and fiber) and mineral (ash) contents except for the moisture content value. We observed that the moisture content increased from 4.5% to 6.81%. The products packed in LDPE packaging material showed a significant change in the proximate content during shelf-life experiments when compared to aluminum packaging material. It increased from 3.4% to 5.4%, while aluminum-packed products increased from 3.4% to 4.82%. This can be explained by the fact that plastic is more susceptible to light, oxygen, and moisture than aluminum packaging and that high moisture content encourages the growth of microorganisms (25).

In most cases, storage temperature reduces the protein content. However, in this study, the weaning food was stored at room temperature, causing fewer changes in the gross protein content. Fat content typically undergoes oxidation during the storage period, leading to rancidity (32). Even so, the presence of vitamin E (more than 50%) reduces the rancidity caused by fat oxidation. It is possible that vitamin E, an antioxidant, reduces fat oxidation, thereby diminishing off-flavor development (27). Carbohydrates are relatively more stable under storage conditions compared to vitamins. There was no significant loss in the nutritional value of carbohydrates during the storage period. The same effect was also noted by Abdullah et al. (28) in dried foods. Distinctively, water-soluble vitamins were more sensitive than fat-soluble vitamins during the storage period (30).

Nevertheless, vitamin B loss during the storage period appeared to be insignificant. However, decreases in the contents of vitamins B1, B2, and C were noticed. These vitamins are highly unstable to light, moisture, and oxygen; subsequently, products packed in LDPE had more vitamin content depletion than those packed with aluminum. There was no detectable alteration in vitamin A content. With regards to mineral content, there was no evident quantifiable variation in this study, which is consistent with other investigations (30).

Reduced viscosity is one of the quality attributes for weaning food preparation (33). Viscosity represents the pasting property of food, which shows no changes in the product's texture. Furthermore, it emphasizes high caloric density per unit volume of food. The formulated freeze-dried weaning food had a low viscosity that slightly rose over storage time. This change was not statistically significant. The preliminary pH of the formulated weaning food was 6.19. It remained acidic during the entire storage period, rising negligibly and never attaining alkalinity. Less acidic foods are easier to digest by infants as their digestive systems are immature (30, 33).

Water activity measures the amount of water present in the product. The higher the water activity, the greater the chance for the growth of microorganisms. In the beginning, the water activity was very low; then, it gradually surged due to adjustments in moisture content (33, 34). In this present study, the overall bacterial count of weaning food was observed to be satisfactory per Food Safety and Standard Authority of India regulations (35). Initially, the count was low, but gradually, it increased, became constant, and then declined. The fungus counts increased during the storage period due to the high moisture content. Fungal growth was more prevalent in the product packed in LDPE (36). A study conducted by Gull et al. (10) discussed the development of weaning food from *ragi* flour, green gram flour, and rice flour and established that *ragi* could be considered a good and cheap source of protein, fat, fiber, calcium, and iron for young children. In their study, they conducted microbial analysis and calculated the standard plate count, which is negligible.

5. Conclusions

Malnutrition is a serious issue and is prevalent worldwide, especially infant malnutrition. The current study demonstrates that both banana and *ragi* freeze-dried weaning formulations can provide the necessary nutrients for an infant's growth and development. The nutritional value of bananas is remarkable due to their high caloric, vitamin, and mineral contents. *Ragi* powder can be used to make porridge for infants and offers several advantages. To boost the nutritional value of the weaning food, adjustments must be made to its formulation, handling, and storage. Overall, the developed weaning food meets RDA values for all necessary nutrients except sodium and vitamin C. The formulated weaning food was fortified with a food-grade premix to improve product quality, and its RDA compliance was confirmed. The selected formulated freeze-dried weaning food was determined to be nutrient-rich and microbially safe for infants. This ready-to-serve weaning food could be considered an effective supplementary food for infants as it consists of natural ingredients and is enriched with micronutrients. These formulation findings may yield a reasonable solution for low-socioeconomic groups.

Data availability statement

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

Author contributions

SK carried out the experiments and wrote the manuscript draft. NG designed, executed, and supervised the study. MQ conducted the content evaluation and critical scientific and technical editing of the manuscript. PR, HA, ME, MQ, and NG intellectually contributed and edited the final 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.

Publisher's note

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Development and bioassessment of high nutria-omega 5 cookies through animal modeling

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The food industry generates a diverse range of waste byproducts during fruit processing, which can be repurposed to create functional foods and other valuable commodities. In this particular study, leftover agro-waste from pomegranate juice was valorized to obtain pomegranate seed oil (PSO), while utilizing sunflower oilseed cake to produce sunflower meal protein concentrate (SMPC). These two extracted components were then combined as ingredients to produce High Nutria Omega 5 (HNO5) cookies. To ensure the quality and viability of pomegranate seed oil, a comprehensive set of laboratory analytical procedures were employed to evaluate its characteristics. Subsequently, different ratios of pomegranate seed oil and sunflower meal protein concentrate were utilized to develop the HNO5 cookie products. These cookies underwent thorough sensory, physicochemical, storage, and proximate evaluations as well as efficacy studies to assess their overall nutritional quality and shelf-life properties. As compared to the control feed, the findings of the renal and liver functional tests indicated a favorable effect on ALT, AST, ALP, serum urea, creatinine, albumin, globulins, total proteins, and A/G ratio. The results revealed that PSO and SMPC cookies containing 15% PSO and 15% SMPC exhibited stability in numerous physicochemical and sensory assessments. The punicic acid in HNO5 cookies significantly reduced the effects of starvation in rats and progressively improved several metabolic processes and overall health profiles.

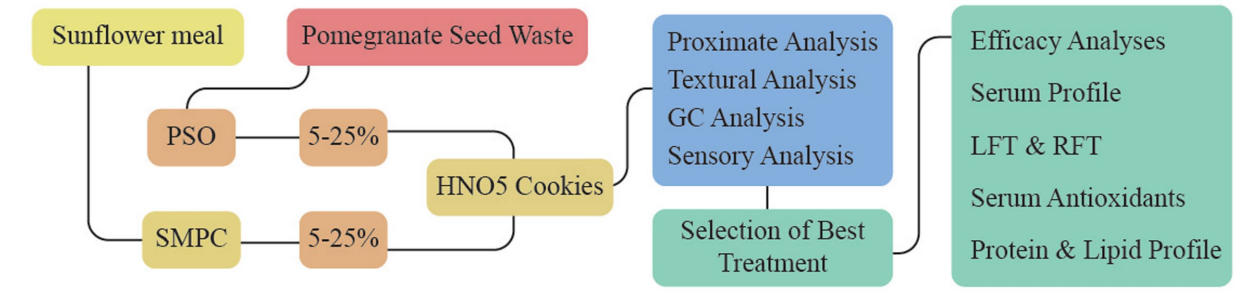
KEYWORDS

agro-industrial waste, malnutrition, high nutria-omega 5 cookies, sunflower meal protein concentrate, pomegranate seed oil

1. Introduction

The pomegranate is one of the oldest fruit plants named "*Punica granatum*," which means "seeded apple" or "grainy apple," driven from the word *Ponum granatum* that has been cited in the Ancient Egyptian documents and the Bible. It is also considered an appealing, pleasant and nutritious fruit due to its rich nutritional properties (1). Pomegranate fruit and its components, such as seed, peel and juice, have a higher antioxidant capacity and other valuable features, motivating scientists to find more latent and beneficial bioactive chemicals for food applications and nutraceuticals (2).

Sunflowers are short-season plants belonging to the genus Helianthus and Asteraceae family, and there are more than seventy classes all over the globe. It originated in the temperate parts of North America (20 to 25°C) and was known to European travelers in the sixteenth century



GRAPHICAL ABSTRACT

(3). Some essential amino acids have also been found in sunflower products, including histidine, threonine, glutamic acid, valine, tyrosine, arginine, methionine, serine, cysteine, alanine, lysine, isoleucine, aspartic acid, glycine phenylalanine and proline (4). Pomegranate has a similar property to *Portulaca oleracea* (purslane), and *Abelmoschus esculentus* (okra) which have wide range of pharmacological effects, including antibacterial, analgesic, anti-inflammatory, and wound-healing qualities. Okra, a member of the Malvaceae family, supplies vital fatty acids for human nutrition in its seed oil. Numerous studies have suggested that okra seed oil can decrease cholesterol. Okra seeds are used to create 20–40% of the world's oil (5, 6).

De-oiled sunflower cake (DC) is a promising and inexpensive protein source due to its high protein content. A long-term plant-based protein manufacturing approach requires the revaluation of industrial waste. There is no report in the literature regarding any toxic or allergic compounds found in sunflower protein (SP) or any genetic alteration. Because of these features, there has been a recent spike in industrial and scientific interest in sunflower protein, particularly for protein extraction or other human ingesting possibilities, such as flour replenishment in bakery items (7, 8). Cookies are small, sweet baked commodities made with flour, eggs, sugar, butter, cooking oil, or other fat. Three basic ingredients are involved in its preparation, i.e., fat, flour and sugar (9).

Malnutrition refers to deficiency or excess of any nutrient in a person's energy intake. Malnourished portions lack nutritious and energetic food, notably lacking protein, vitamins and trace minerals. Pakistan is facing malnutrition that disproportionally disturbs almost all females. People belonging to low-income families are more prone to malnutrition and infectious diseases. The prevalence of malnutrition (underweight, stunting, and wasting) increases children's mortality risk. Vitamin and iron inadequacy are micronutrient-related malnutrition (10).

Considering the facts mentioned earlier, pomegranate seed oil and sunflower meal protein concentrate can be valuable ingredients in ameliorating malnutrition. The functional properties of punicic acid and protein isolates obtained through extraction used in the formulation of High nutria omega 5 (HNO5) cookies were also estimated. For bio-evaluation, tests were performed on experimental rats to determine the influence of the resulting cookies on their suitability, efficiency, and scope of use. The primary goals of this study encompassed the utilization of waste sunflower and pomegranate seeds as valuable resources for preparing value-added ingredients. Additionally, the study aimed to create high nutria-omega 5 cookies with enriched properties of SMPC and PSO while also investigating their storage stability. Furthermore, an efficacy study was conducted to evaluate the potential health benefits of consuming these high nutria-omega 5 cookies.

2. Materials and methods

The discarded pomegranate seeds and sunflower meal were obtained from a local seller in Faisalabad, Pakistan.

2.1. Oil extraction

The oil was extracted using the Soxhlet extraction technique from the clean and dry powder of pomegranate seeds, as described by Jing et al. (11). The sample was dried and added to a thimble; n-hexane solvent was used for Soxhlet extraction. The obtained pomegranate seed oil (PSO) was purified through rotary evaporation and kept at −20°C in amber-colored bottles for further evaluation.

2.2. Fatty acid composition

PSO was converted to fatty acid methyl esters (FAME) using the method described and adopted by Khoddami et al. (12) and Ledoux et al. (13), with some changes. A 50 mg oil sample was trans-esterified with 50 µL sodium methoxide and 950 µL n-hexane. After vortexing for 5 s and settling for 5 min, a 1 µL aliquot of the top layer was taken for Gas Chromatograph (GC) analysis.

2.3. Preparation of sunflower meal protein concentrate

Milled sunflower meal was concentrated according to the procedure described by Salgado et al. (14) and Lovatto et al. (15) with some modifications. Sunflower oilseed cake (67 g/L) was mixed with water and stirred for 1 h at pH 9 using NaOH (3 mol/L). The mixture was then separated using a basket type centrifuge at 2100 G (RCF) and 20°C for 30 min. The resulting supernatants from protein extraction were combined and an isoelectric precipitation was conducted by adding 3 mol/L until reaching pH 9.

2.4. Crude protein

The crude protein contents of SMPC were estimated according to Kjeldahl's method as described in AACC (2000) Method No. 46-10.

2.5. Amino acid characterization

The amino acid profiling of SMPC was performed with the help of an amino acid analyzer. The sample preparation was done according to the method recommended by EU Directive (16).

2.6. Product development

The cookies were prepared according to the procedure described in AACC (17) Method No. 10-50d. According to the formulation of various treatments, cookies were designed using different concentrations of sunflower meal protein concentrate (Supplementary Table S1).

2.7. Analysis of high nutria omega 5 cookies

The prepared High Nutria Omega 5 cookies were subjected to various quality analyses, and their storage stability was investigated at different storage intervals (0, 15, 30, 45, and 60 days).

2.7.1. Crude protein

The crude protein content of sunflower protein concentrate samples was determined using the Kjeldahl's method No. 46-10 is mentioned in AACC (17).

2.7.2. Textural analysis

Following the method described by Chauhan et al. (18), the textural parameter was calculated using a 5 kg load cell texture analyzer (Model TA-XT2, Secure Microsystems and Surrey, UK).

2.7.3. GC analysis

The fatty acid analysis was performed as described in Section 2.2.

2.7.4. Energy value

The energy value of HNO5 cookies was calculated using the Oxygen Bomb Calorimeter (IKA-WERKE, C2000 Basic, GMBH and Co., Germany), as defined by Krishna and Ranjhan (19).

2.7.5. Sensory evaluation of cookies

The taste panel scored the cookies of various treatments using the 9-point hedonic scoring scale (20).

2.7.6. Selection of best treatment of cookies

The best treatment was selected based on storage stability and sensory evaluation for the efficacy study.

2.8. Experimental plan

An *in-vivo* study was conducted using 50 eight-week-old Sprague Dawley rats, weighing an average of 280 ± 10 g. The rats were obtained from the National Institute of Food Science and Technology. They were housed in metabolic cages at room temperature (25°C) with a 12:12 light–dark cycle. The study followed the guidelines for animal care and use, approved by the Institutional Biosafety/Bioethics Committee. The rats were acclimated to the environment and provided with a regular diet and water *ad libitum* for three days. For the experiment, the rats were randomly divided into five groups, each consisting of 10 rats (Supplementary Table S2). The ingredients of all experimental diet plans are shown in Supplementary Table S3. The first group served as the reference and received a standard diet throughout the experiment. The other four groups of rats underwent eight days of long-term starvation, resulting in weight loss (average of 190 ± 10 g). After starvation, the G0 group received a basal diet for 45 days, while G1 and G2 were given 15% control and 15% HNO5 (high fat and high protein) cookies incorporated into their feed for the same period. The diets for G3 and G4 were equivalent to the protein and fat ratios found in HNO5 cookies. The experimental diets were compared to the control groups (G0 and G1). The study aimed to assess the specific benefits of PSO, SMPC, and HNO5 cookies by re-feeding the starved rats. Evaluations were conducted at 0, 15, 30, and 45 days after the rats were re-fed following the eight-day starvation period. On the final day, the animals were euthanized for further analysis.

2.9. Analysis of serum profile

According to the standard method, the serum from the blood sample was separated using the centrifuged machine for serum analyses.

2.9.1. Level of punicic acid

Punicic acid levels in serum were determined when the lipids were extracted from rat serum using the method Yuan et al. (21) explicated.

2.9.2. GC analysis

The FAMEs were analyzed on a GC column system according to the protocol illustrated by Benner et al. (22) with some modifications. A GC system with a flame ionization detector was utilized, equipped with a biscyanopropyl polysiloxane capillary column (100 m × 0.25 mm × 0.25 µm). The injection port and the detector were kept at 250°C. FAME peaks were identified by comparing their retention time to a reference standard. Punicic acid content was expressed as a percentage of fatty acids in lipid fractions.

2.9.3. Serum proteins

Following the manufacturer's instructions, commercially available kits (Bioclin® Total Protein Monoreagent Diagnostic Kit; K031 and Bioclin® Albumin Monoreagent Diagnostic Kit; K040) were used to assess total protein and albumin concentrations in serum samples. The serum globulin concentration in each piece was calculated by subtracting the serum albumin concentration from the total protein concentration.

2.9.4. Protein quality evaluation

The protein efficiency ratio (PER) was determined by following the equation developed by Henry (23), which corresponded to the

ratio of protein intake and body weight. Similarly, the net protein ratio (NPR) was calculated using body weight loss observed through a protein-free diet in the control cookies group by following the equation described by Bender and Doell (24).

2.10. Serum antioxidants

The total antioxidant capacity (TAC) in the serum samples and serum total oxidative stress (TOS) were determined using the novel automated calorimetric approach as described by Erel (25).

2.11. Lipid profile

Serum concentrations of total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were measured using the Trinder enzymatic method using Liquiform Cholesterol kit (Lab test Diagnostics, Brazil).

2.12. Statistical analysis

The collected data was finalized, and outcomes from contemporary research were statistically analyzed by applying a complete randomized design (CRD) with Tukey's HSD *post hoc* test for further analysis according to the protocol defined by Montgomery (26).

3. Results and discussions

The objective of the present study was to characterize the significance of pomegranate seed oil (PSO) and sunflower meal protein concentrate (SMPC) with regards to nutritional and functional properties of the developed edible product (High nutria Omega-5 (HNO5) cookies) to treat malnutrition related consequences.

3.1. Fatty acid composition of pomegranate seed oil

The results regarding the fatty acid profile are shown in Table 1. The major fatty acid found in oil samples was conjugated linolenic acid (CLnA, C18:3), known as punicic acid, one of PSO's principal and active components responsible for its antioxidant potential (27).

TABLE 1 Concentrations for fatty acid composition in PSO (Wt. %).

PSO	Concentration
Myristic acid (C14:0)	0.31
Palmitic acid (C16:0)	5.12
Stearic acid (C18:0)	2.65
Oleic acid (C18:1)	10.24
Linoleic acid (C18:2)	5.45
Punicic acid (C18:3)	73.21
SFA	8.29
UFA	90.12

Conjugated linolenic acid was found as abundant as 73.21%. The other fatty acids observed included linoleic acid (CLA, C18:2), which was 5.45%, followed by oleic acid (C18:1), 10.24%. However, myristic (C14:0), stearic (C18:0) and palmitic (C16:0) acids were found in lesser extent, i.e., 0.31, 2.65, and 5.12%, respectively. Total amount of saturated fatty acids (SFAs) was 8.29%, whereas unsaturated fatty acids (MUFAs and PUFAs) was 90.12%. The presence of punicic acid is linked to the biological and health benefits of pomegranate seed oil (28). According to Siano et al. (29), punicic acid is stable at 50°C; heating it at 170°C for 4 h causes weak isomerization into positional and geometrical isomers.

3.2. Crude protein of sunflower meal protein concentrate

The protein content of SMPC was found to be 55.19 ± 1.27%. In a similar study, Gandhi, Jha (30) observed sunflower meal to have 57.4% protein.

3.3. Amino acid characterization

The amino acid profile of SMPC was shown in Supplementary Table S4, in which arginine was 2.59%, histidine 0.97%, isoleucine 1.35%, leucine 1.96%, lysine 0.99%, methionine 0.44%, cysteine 1.22%, phenylalanine 1.55%, threonine 1.85%, valine 1.19%, aspartic acid + asparagine 1.09%, serine 0.49%, glutamic acid + glutamine 1.37%, glycine 0.45%, alanine 0.48%, tyrosine 0.56% and proline was 1.05%. The amino acid profiles may vary based on cultivar varieties, industrial processes and crop/pest control methods (31). While studying the bioactivity potential of industrial sunflower protein ethanol-wash solute, Ivanova, Ivanov (32) observed that leucine and isoleucine had the highest value of all essential amino acids, at 1.16 and 1.40%, respectively.

3.4. Proximate analyses of high nutria omega 5 cookies

The higher protein content of 13.36 ± 0.22% was seen in T_5 (25% SMPC and 25% PSO), while the lower value of 7.09 ± 0.12% was found in T_0 at 0 days, which diminished as the extent of SMPC and PSO diminished with storage days (Table 2). The results proved that SMPC and PSO show significantly more protein percentages than the control. In a similar study, the protein content of sunflower meal protein concentrates was 57.4%, as described by Gandhi, Jha (30) and 19.60% protein in PSO. In newly prepared cookies, the protein percentage significantly reduced between 6.98 and 10.93% following 60 days ($p \leq 0.05$). This reduction in the protein substance of cookies amid storage is because of the retention of dampness from the environment. The different proteins found in flour can alter during food processing and storage due to protein cross-linking, protein-carbohydrate interactions, and protein denaturation. Non-enzymatic reactions may also result in food deterioration and shorten the shelf life of foods (33). The results from Table 3 SI indicate that SMPC and PSO fortification may help improve protein content status.

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TABLE 2 Means for the effect of treatment and storage intervals on the proximate parameters of high nutria cookies.

Proximate parameters	Treatment	Days				
		0	15	30	45	60
Protein content (%)	T_0	7.09 ± 0.12	7.07 ± 0.08	7.03 ± 0.08	7.01 ± 0.03	6.98 ± 0.03
	T_1	7.39 ± 0.11	7.32 ± 0.12	7.18 ± 0.12	7.14 ± 0.10	7.11 ± 0.08
	T_2	9.36 ± 0.12	9.28 ± 0.13	9.21 ± 0.10	9.19 ± 0.12	9.13 ± 0.15
	T_3	10.64 ± 0.24	10.49 ± 0.22	10.48 ± 0.14	10.36 ± 0.08	10.35 ± 0.13
	T_4	11.58 ± 0.24	11.11 ± 0.18	10.84 ± 0.22	10.76 ± 0.12	10.48 ± 0.19
	T_5	13.36 ± 0.22	12.97 ± 0.22	12.39 ± 0.25	11.53 ± 0.22	10.93 ± 0.24
Fat content (%)	T_o	15.32 ± 0.26	15.17 ± 0.27	15.12 ± 0.34	15.06 ± 0.35	14.95 ± 0.26
	T_1	15.51 ± 0.33	15.36 ± 0.23	15.23 ± 0.22	15.30 ± 0.25	15.24 ± 0.42
	T_2	16.76 ± 0.32	16.66 ± 0.23	16.56 ± 0.28	16.49 ± 0.36	16.42 ± 0.24
	T_3	17.67 ± 0.38	17.54 ± 0.24	17.56 ± 0.25	17.52 ± 0.27	17.41 ± 0.36
	T_4	18.72 ± 0.34	18.41 ± 0.39	18.11 ± 0.37	18.14 ± 0.26	17.89 ± 0.33
	T_5	19.42 ± 0.35	19.33 ± 0.32	19.13 ± 0.31	18.99 ± 0.47	18.91 ± 0.27
Textural hardness (N)	T_0	18.05 ± 0.71	17.87 ± 0.71	17.55 ± 0.30	16.92 ± 0.59	14.85 ± 0.56
	T_1	14.81 ± 0.49	14.53 ± 0.28	14.43 ± 0.27	14.15 ± 0.62	13.87 ± 0.51
	T_2	17.55 ± 0.38	17.17 ± 0.47	16.95 ± 0.36	16.65 ± 0.45	15.76 ± 0.44
	T_3	20.18 ± 0.26	19.65 ± 0.46	19.26 ± 0.32	19.10 ± 0.74	18.72 ± 0.45
	T_4	21.82 ± 0.29	21.68 ± 0.59	21.21 ± 0.51	20.96 ± 0.64	20.73 ± 0.62
	T_5	24.98 ± 0.37	24.62 ± 0.56	24.53 ± 0.37	24.34 ± 0.72	23.82 ± 0.62
Spread factor	T_o	52.32 ± 0.66	52.52 ± 0.54	52.62 ± 0.59	52.64 ± 0.59	52.59 ± 0.55
	T_1	53.33 ± 0.68	53.73 ± 0.61	53.82 ± 0.71	53.87 ± 0.59	53.92 ± 0.59
	T_2	53.15 ± 0.67	54.22 ± 0.59	55.08 ± 0.61	54.81 ± 0.74	55.01 ± 0.52
	T_3	54.27 ± 0.71	54.51 ± 0.66	54.44 ± 0.67	55.28 ± 0.66	55.38 ± 0.55
	T_4	54.54 ± 0.51	54.28 ± 0.63	54.34 ± 0.67	54.92 ± 0.59	54.80 ± 0.63
	T_5	54.94 ± 0.62	54.17 ± 0.64	53.75 ± 0.52	54.42 ± 0.64	54.17 ± 0.53
Energy value (kcal/100 g)	T_o	439.5 ± 9.6	438.8 ± 10.1	431.4 ± 11.1	425.7 ± 10.2	423.5 ± 11.5
	T_1	394.2 ± 8.8	392.6 ± 11.4	390.5 ± 11.7	385.4 ± 9.3	386.3 ± 10.5
	T_2	417.8 ± 11.9	414.6 ± 10.3	409.3 ± 10.2	408.1 ± 11.7	402.2 ± 9.3
	T_3	443.6 ± 10.5	440.2 ± 10.7	439.2 ± 11.7	435.5 ± 11.1	432.2 ± 11.8
	T_4	474.4 ± 10.5	474.9 ± 11.7	470.8 ± 8.9	468.2 ± 10.2	467.3 ± 11.9
	T_5	494.9 ± 9.5	495.0 ± 8.8	493.9 ± 9.9	491.1 ± 10.5	487.4 ± 8.9

TABLE 3 Mean values for protein quantity evaluation in rats.

Groups	Total food intake (g)	Weight gain (g)	Protein in feed (%)	Protein consumed (g)	Protein Efficiency Ratio	Net Protein Ratio
G_0	616.23 ± 25.72	101.96 ± 09.72	19.37 ± 0.54	119.31 ± 5.66	0.85 ± 0.08	0.76 ± 0.10
G_1	473.76 ± 16.32	−10.96 ± 03.43	0.17 ± 0.04	0.80 ± 0.05	NA	NA
G_2	529.55 ± 28.11	160.02 ± 12.72	21.42 ± 0.62	113.31 ± 8.36	1.41 ± 0.09	1.31 ± 0.13
G_3	548.32 ± 32.88	188.68 ± 16.92	24.36 ± 1.02	133.49 ± 7.53	1.66 ± 0.11	1.33 ± 0.09
G_4	501.71 ± 21.67	97.32 ± 08.87	19.57 ± 0.38	98.04 ± 4.55	0.99 ± 0.06	0.88 ± 0.07

3.5. Crude fat of high nutria omega 5 cookies

The mean values of fat content (%) in High Nutria Omega 5 cookies with different levels of SPMC and PSO are shown in Table 2. A gradual decline was observed in the fat content of the treatments as the storage time increased. This effect was most notably observed in T_5, where the fat content reduced from 19.42 ± 0.35% at 0 days to 18.91 ± 0.27% at 60 days of storage time. This decline was less aggressive in treatments with lower PSO and SMPC levels. Since PSO has a high amount of

polyunsaturated fatty acids and shortening mostly has a higher amount of saturated fats, this may explain why the declination was more linear in treatments with a higher amount of PSO owing to the lower stability of unsaturated fats. A significant variation level was observed among treatment and storage groups ($p \leq 0.05$). According to Sharif, Butt (34), this reduction in crude fat during the storage of cookies may be due to the absorption of humidity in cookies from the climate and the oxidation of unsaturated fats breaking down the free unsaturated fat arrangement.

3.6. Textural analysis

Textural profile is an imperative parameter for assessing physical nature of food especially baked goods. The means of high nutria cookies for the texture of different treatments appeared in Table 2. The textural hardness was observed to significantly decrease as the storage days increased ($p \leq 0.05$), which may be attributed to the incorporation of moisture from the atmosphere leading to an increase in moisture content and eventually adding to the dampness of the cookies, which may have gradually reduced the hardness of the cookie treatments. A similar study by Giuffrè et al. (35) also revealed that Cantuccini Biscuits prepared by replacement of shortening with extra virgin olive oil had a decreasing trend in both hardness and fracturability value over 12 months.

3.7. Spread factor

The spread factor varied from 52.32 ± 0.66 to 55.38 ± 0.55 in cookies of all treatments. Results reveal that the spared factor of high nutria cookies showed significant variation ($p \leq 0.05$) with a rise in SMPC and PSO levels for each treatment. The means for the spread factor of baked cookies are mentioned in Table 2. Ahmad et al. (36) observed a similar trend of values while studying the effect of additives on gluten development in cookie dough. The spread factor of cookies from different mills was 40.35 ± 0.620 and 57.66 ± 0.543. Chappalwar et al. (37) reported the same result but observed no consistent trend in the spread factor of the products. The result of this research is in relation to studies explicated by Claughton and Pearce (38), where they detected a slightly non-significant increase in the spread factor of HNO5 equipped from flour comprising 20% sunflower isolate.

3.8. Energy value

The energy value decreased significantly ($p \leq 0.05$) as the storage time elapsed to 60 days. Overall, the lowest energy value was observed in T_1 (386.3 ± 10.5 kcal/100 g), and the highest energy value was observed in T_5 (487.4 ± 8.9 kcal/100 g) after 60 days as shown in Table 2. This apparent decline in energy value may be attributed to the oxidation of oil in cookies and moisture gain from the surrounding resulting in lower protein content.

3.9. Characterization of conjugated linolenic acid (Omega-5 punicic acid)

In the freshly prepared cookies, the content of punicic acid ranged from 3.77 to 16.14% on the 0th day. Over a period of 60 days, this percentage gradually decreased to a range of 3.33 and 14.37%. As apparent from the mean values in Supplementary Table S5, a significant decline in the punicic acid of high nutria cookies can be seen after a storage time of 60 days. A considerable variation was observed in both treatment levels and the storage interval of the High Nutria Omega 5 cookies ($p \leq 0.05$). According to Sharif et al. (39), a reduction in fatty acid contents during the storage of cookies could result from moisture absorption in cookies from the environment and oxidation of unsaturated fats bringing about free fatty acids. Punicic acid is generally known as CLnA (conjugated linolenic acid) and PSO generally contains 78% punicic acid (40).

3.10. Selection of best treatment

The consequent data obtained from the proximate, physicochemical, storage and sensory evaluation revealed that T_3 (15% PSO + 15% SMPC) was most suitable for further evaluation (Supplementary Figures S1–S5).

3.11. Serum analyses

3.11.1. Liver functioning test

In our investigation, malnourished groups of rats had significantly higher blood levels of the liver function indicators ALT, AST, and ALP than normal control rats. The rationale for the significant increase in serum levels of aminotransferases in the current study's starved rats may be that these enzymes escaped into circulation following hepatic damage in the context of prolonged fasting (41). According to the results, administering several experimental diets considerably improved the blood levels of the enzymes ALT, AST, and ALP (Table 4). These reported benefits might result from SMPC and PSO's flavonoids, terpenoids, and alkaloids, which have antioxidant and hepatoprotective activities. Along with their ability to stabilize membranes, these phyto-constituents are known to inhibit the release of intracellular liver enzymes. These outcomes are backed up by one of the earlier investigations, which used high carbohydrate, high protein, and high-fat diets, which were re-fed to rats after 3 days of starvation. After following a high fat and high protein diet, the levels of ALT and AST returned to the normal range as per their respective reference values. However, in the case of a high carbohydrate diet, liver functioning tests showed significantly higher values compared to the reference values (42).

3.11.2. Renal functioning tests

Results have shown that mean urea and creatinine in malnourished rats were significantly increased after starvation (G_1). Treatment of malnourished rats with HNO5 cookies, SMPC and PSO significantly decreased the level of urea and creatinine from day 0 to the 45th day of the experiment (Table 5). Moreover, experimental diets in treated groups G_2, G_3, and G_4 from day 0 to day 45 also restored the renal functioning in the reference range affected due to malnutrition compared to the control group (G_0). This finding is consistent with previous research involving starved rats to determine suitable diet recovery with macronutrients.

Creatinine and urea are metabolism's nitrogenic end products. Urea is the primary metabolite of the recycling of tissue and dietary protein

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TABLE 4 Means for the effect of groups and days on the liver functioning tests of rats.

	Group	Days			
		0	15	30	45
ALT (U/L)	G_0	39.41 ± 1.78	40.01 ± 1.22	39.12 ± 1.79	38.37 ± 1.89
	G_1	44.45 ± 1.83	43.39 ± 1.04	42.62 ± 1.52	42.76 ± 1.98
	G_2	45.37 ± 1.78	42.02 ± 1.78	38.62 ± 1.93	36.32 ± 1.15
	G_3	44.20 ± 1.66	41.62 ± 1.01	37.18 ± 1.06	34.46 ± 1.63
	G_4	45.14 ± 1.59	43.12 ± 1.77	40.09 ± 1.12	38.31 ± 1.97
AST (U/L)	G_0	135.21 ± 02.98	134.35 ± 02.43	134.05 ± 02.98	133.97 ± 02.73
	G_1	142.50 ± 02.84	142.35 ± 02.49	141.19 ± 03.04	141.07 ± 03.04
	G_2	141.03 ± 01.27	137.41 ± 02.44	134.37 ± 02.31	131.20 ± 02.37
	G_3	142.25 ± 01.59	140.41 ± 02.79	137.04 ± 02.15	135.43 ± 02.52
	G_4	143.09 ± 03.56	142.95 ± 03.14	138.63 ± 02.42	132.19 ± 02.60
ALP (U/L)	G_0	54.63 ± 1.89	51.47 ± 1.39	53.45 ± 1.44	52.42 ± 1.98
	G_1	64.54 ± 1.91	63.70 ± 1.97	61.78 ± 1.45	59.47 ± 1.09
	G_2	65.61 ± 1.14	62.61 ± 1.42	60.43 ± 1.11	57.60 ± 1.77
	G_3	64.59 ± 1.15	61.27 ± 1.38	59.62 ± 1.06	54.51 ± 1.46
	G_4	63.68 ± 1.65	62.65 ± 1.79	58.68 ± 1.86	55.68 ± 1.31

TABLE 5 Means for the effect of groups and days on the renal functioning tests of rats.

	Group	Days			
		0	15	30	45
Urea level (mg/dL)	G_0	22.12 ± 0.60	21.93 ± 0.56	21.41 ± 0.49	21.08 ± 0.54
	G_1	25.97 ± 0.54	26.01 ± 0.26	24.64 ± 0.91	24.30 ± 0.49
	G_2	26.40 ± 0.49	25.03 ± 0.97	22.31 ± 0.39	20.65 ± 0.41
	G_3	26.08 ± 0.91	25.12 ± 0.27	23.16 ± 0.44	21.06 ± 0.63
	G_4	25.94 ± 0.56	24.78 ± 0.47	22.78 ± 0.32	20.43 ± 0.44
Creatinine level (mg/dL)	G_0	0.55 ± 0.011	0.52 ± 0.012	0.51 ± 0.010	0.49 ± 0.013
	G_1	0.84 ± 0.016	0.82 ± 0.012	0.81 ± 0.016	0.80 ± 0.013
	G_2	0.80 ± 0.012	0.75 ± 0.014	0.68 ± 0.019	0.58 ± 0.010
	G_3	0.85 ± 0.015	0.73 ± 0.010	0.68 ± 0.004	0.56 ± 0.002
	G_4	0.66 ± 0.013	0.58 ± 0.008	0.51 ± 0.009	0.54 ± 0.004

(43). Factors like dehydration and low-protein diets are responsible for an increased level of urea, whereas creatinine is more particular to the kidney (44). Hence, research findings show that administration of PSO, SMPC and particularly combined in HNO5 cookies inhibited serum urea and creatinine while enhancing renal function.

3.11.3. Level of punicic acid

Results indicated a significant increase in serum punicic acid with respect to days and treatments. Increased levels of punicic acid were observed in groups G_2 and G_4 at the end of the study, with levels of 0.43 and 0.46% of fatty acids, respectively. At the same time, G_0, G_1 and G_3 had no value of punicic acid observed as there was no source of punicic acid in their diets designed (Figure 1). Punicic acid significantly ($p \leq 0.05$) varied among different groups. These results are also supported by a study by Yuan et al. (2009) on punicic acid metabolism and its retaining in blood in young, healthy males. In another study, 30 males were randomly divided into two groups after 7 days of adaptation period with sunflower seed kernels. The control group was fed sunflower seed kernels, while the second group was with punicic acid rich *Trichosanthes kirilowii* (TK) seeds with 3 g of punicic acid, and the study period was for 28 days. Serum punicic acid was evaluated at the start and end of the trial in both groups. Punicic acid was zero on the first day of study in all the experimental groups; however, it raised significantly in the HNO5 cookies group, and PSO treated group with punicic acid-rich seeds but remained 0 in the normal and positive control group. It was incorporated into human tissues, and some of it was metabolized into cis9, trans11-18:2.

3.11.4. Serum proteins

Long-term starvation significantly reduced the metabolic stress indicators such as total serum proteins, albumin, globulin, and A/G ratio in malnourished rats compared to positive control and negative control (normal group; Table 6). The ratio of protein production to catabolism inside the body determines the number of tissue proteins.

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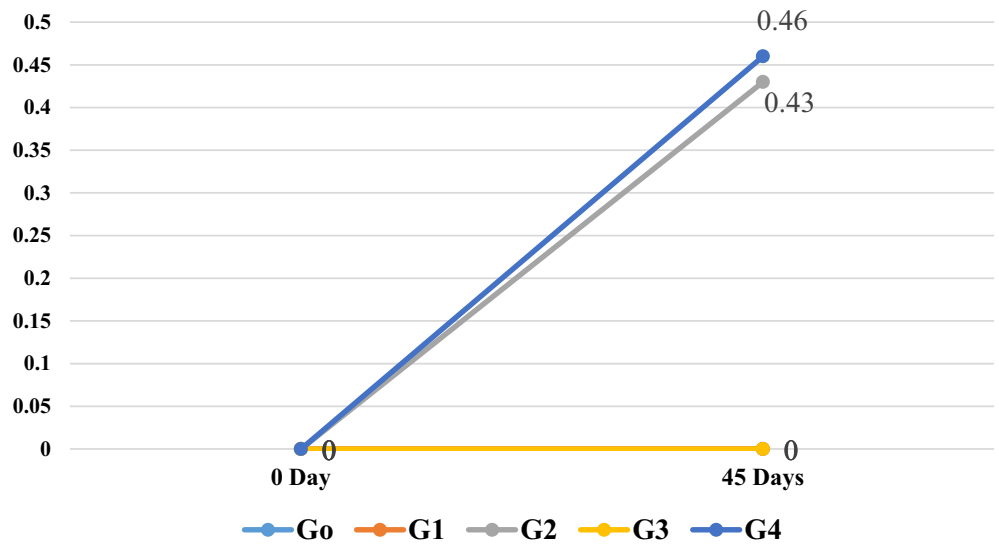

FIGURE 1
Effect of groups and days on the serum punicic acid (% fatty acid) in rats.

TABLE 6 Means for the effect of groups and days on the serum proteins of rats.

	Group	Days			
		0	15	30	45
Total serum proteins (g/dL)	G_0	5.92 ± 0.23	5.93 ± 0.15	5.94 ± 0.13	5.96 ± 0.14
	G_1	5.06 ± 0.12	4.97 ± 0.17	4.90 ± 0.19	4.85 ± 0.17
	G_2	5.04 ± 0.14	5.97 ± 0.26	6.44 ± 0.13	7.03 ± 0.18
	G_3	5.07 ± 0.15	5.93 ± 0.13	6.81 ± 0.14	7.62 ± 0.25
	G_4	5.06 ± 0.16	5.04 ± 0.15	5.02 ± 0.16	5.01 ± 0.14
Serum albumin (g/dL)	G_0	3.71 ± 0.06	3.72 ± 0.04	3.74 ± 0.03	3.76 ± 0.01
	G_1	2.21 ± 0.02	2.15 ± 0.05	2.09 ± 0.01	2.05 ± 0.04
	G_2	2.20 ± 0.04	2.85 ± 0.07	3.18 ± 0.02	3.82 ± 0.08
	G_3	2.24 ± 0.07	2.87 ± 0.06	3.34 ± 0.09	3.92 ± 0.07
	G_4	2.22 ± 0.03	2.21 ± 0.05	2.19 ± 0.04	2.18 ± 0.02
Serum globulins (g/dL)	G_0	2.21 ± 0.18	2.21 ± 0.13	2.20 ± 0.15	2.20 ± 0.15
	G_1	2.85 ± 0.13	2.82 ± 0.15	2.81 ± 0.13	2.80 ± 0.19
	G_2	2.84 ± 0.19	3.12 ± 0.14	3.26 ± 0.16	3.21 ± 0.13
	G_3	2.83 ± 0.14	3.06 ± 0.19	3.47 ± 0.11	3.70 ± 0.11
	G_4	2.84 ± 0.12	2.83 ± 0.11	2.83 ± 0.14	2.83 ± 0.15
A/G ratio	G_0	1.67 ± 0.01	1.68 ± 0.01	1.70 ± 0.02	1.71 ± 0.09
	G_1	0.77 ± 0.02	0.76 ± 0.02	0.74 ± 0.01	0.73 ± 0.02
	G_2	0.77 ± 0.02	0.91 ± 0.09	0.97 ± 0.09	1.19 ± 0.02
	G_3	0.79 ± 0.09	0.93 ± 0.01	0.96 ± 0.01	1.05 ± 0.01
	G_4	0.78 ± 0.01	0.78 ± 0.02	0.77 ± 0.02	0.77 ± 0.01

Increased protein catabolism may cause a drop in total proteins and albumin levels (45). According to the findings of our study, experimental diets restored serum total proteins, albumin, globulin, and the A/G ratio compared to the positive control group. Compared to the positive control, the experimental diets HNO5 cookies and SMPC restored the blood total protein level more successfully. Protein content in SMPC and HNO5 cookies has been determined to be 53.19 and 10.35%, respectively, and might aid in restoring the blood total protein level. Malnourished rats given with SMPC and HNO5 cookies had higher blood total proteins, albumin, globulin, and A/G ratios, which may have been caused by insulin-stimulated amino acid uptake, enhanced protein synthesis, and decreased protein breakdown, and a similar trend has been observed by Vidhya and Udayakumar (46).

3.11.5. Protein quality evaluation

The protein efficiency ratio is the gain in body weight per unit protein intake, and the net protein ratio is defined as the ratio of the sum of weight gain and average weight loss to that of protein intake of the test protein group after 28 days. Table 3 shows mean squares for the (NRP) protein quality and protein efficiency ratio (PER) of the test diets. All protein quality parameters (PER and NPR) varied significantly across the experimental diets. Apart from the control, SMPC-based diets (G_2 and G_3) had the highest values compared to the PSO containing diet after 45 days of study duration. Improved protein quality was observed in experimental diets containing SMPC. Rats on a diet devoid of protein underperformed regarding growth, food intake, and weight loss. The same conclusions were reported by Ekpo (47) as both protein consumption and food intake significantly influence growth while low protein leads to decreased food intake and reduced development. However, according to other research, animals given ordinary maize exhibited consistent development (48). Halimatul et al. (49) also observed a similar increasing trend in PER and NPR of Sprague Dawley rats that were fed with Roselle (*Hibiscus sabdariffa* L.) seeds.

3.11.6. Lipid profile

Overall, lipid profile measurements showed a statistically significant difference between all treatment groups after study

completion. Diets containing PSO and HNO5 cookies reduced cholesterol significantly compared to control cookies. Concerning serum lipids profile, malnourished rats presented different TC results depending on the experimental diet containing high protein (G_3) than the positive control (G_1) protein-free group. Although, chronic malnutrition causes endocrine changes in metabolic profile disorders. The decrease in triglyceride level in the HNO5 cookies group compared to control cookies is shown in Table 7. Triglyceride levels of rat's lipid profile in HNO5 and PSO treated groups were significantly improved, indicating that the oil is a potent cardio-protective treatment. This effect was attributed to the super conjugated linolenic acid, or punicic acid, that is contained in pomegranate seed oil. Widyastuti et al. (50) revealed that rats with PEM had higher levels of total cholesterol, triglycerides, and LDL, whereas their HDL levels dropped when compared to the healthy control group. HDL cholesterol levels were significantly increased in rats fed on SMPC and PSO diet with a significant decrease in LDL cholesterol levels. Pasqua et al. (51) also delineated results for evaluating the safety and beneficial outcomes of a conventional diet supplemented with whey-derived protein puddings and hemp seed oil to counteract malnutrition; no significant variations were discovered in plasma total, LDL and HDL cholesterol as well as in glycaemia.

3.11.7. Serum antioxidants

Statistical analysis revealed significant ($p \leq 0.05$) variation in groups and with respect to study duration on serum TAC content. Endogenous antioxidant enzymes significantly affect the body's defense against free radicals, and increased total antioxidant status after being treated with experimental diets ensures a safe and effective strategy in alleviating oxidative stress, most probably due to PSO antioxidant potential. Ali et al. (52) also determined that in both usually fed (NF) and protein-malnourished rats, treatment of AlCl3 dramatically raised MDA and lowered SOD and TAC activity. However, compared to NF rats, protein-malnourished rats had a significantly higher level of MDA and a lower level of SOD and TAC activity (Table 8).

4. Conclusion

the incorporation of pomegranate seed oil (PSO) and sunflower meal protein concentrate (SMPC) in the production of High Nutria Omega 5 (HNO5) cookies has yielded promising results. The study demonstrated that cookies containing 15% PSO and 15% SMPC displayed stability in various physicochemical and sensory evaluations. Furthermore, the inclusion of punicic acid from PSO and SMPC in the HNO5 cookies showed significant improvements in overall health and reduced the negative effects of starvation in rats, leading to enhanced body weight and overall well-being. The positive outcomes of this study indicate that these byproducts can be valuable resources in addressing malnutrition, potentially replacing commonly used ingredients like vegetable shortening and flour. By utilizing these waste byproducts in the food industry, a

TABLE 7 Means for the effect of groups and days on the lipid profile of rats.

	Group	Days			
		0	15	30	45
Total Cholesterol (mg/dL)	G_0	59.71 ± 1.99	60.67 ± 1.96	59.54 ± 2.01	58.57 ± 1.97
	G_1	67.34 ± 1.39	70.36 ± 1.87	76.38 ± 2.08	87.45 ± 2.07
	G_2	69.02 ± 1.41	66.68 ± 1.07	64.70 ± 1.14	62.23 ± 1.87
	G_3	69.56 ± 1.20	65.38 ± 1.86	63.27 ± 1.09	60.27 ± 1.02
	G_4	68.27 ± 1.71	66.15 ± 2.00	62.21 ± 1.19	59.04 ± 1.07
Triglyceride (mg/dL)	G_0	73.45 ± 1.74	72.09 ± 1.31	72.14 ± 1.09	71.50 ± 1.96
	G_1	67.40 ± 1.46	69.34 ± 1.37	73.19 ± 1.57	77.25 ± 1.12
	G_2	76.24 ± 1.35	74.27 ± 1.08	72.25 ± 1.75	69.36 ± 1.77
	G_3	78.65 ± 1.60	76.32 ± 1.06	74.56 ± 2.09	72.63 ± 1.43
	G_4	76.52 ± 1.26	73.06 ± 1.16	70.28 ± 1.51	66.58 ± 1.42
LDL (mg/dL)	G_0	50.30 ± 1.07	48.42 ± 1.47	49.29 ± 1.61	47.40 ± 1.50
	G_1	59.21 ± 1.60	62.36 ± 1.62	65.21 ± 1.16	68.23 ± 1.63
	G_2	58.15 ± 1.66	56.28 ± 1.37	55.17 ± 1.34	54.27 ± 1.52
	G_3	59.04 ± 1.25	57.34 ± 1.78	54.09 ± 1.63	51.36 ± 1.90
	G_4	58.36 ± 1.75	56.20 ± 1.42	53.45 ± 1.04	49.48 ± 1.68
HDL (mg/dL)	G_0	44.75 ± 1.39	45.15 ± 1.68	46.16 ± 1.29	45.13 ± 1.22
	G_1	40.67 ± 1.53	38.23 ± 1.37	37.21 ± 1.36	35.03 ± 1.65
	G_2	40.45 ± 1.57	41.09 ± 1.93	42.32 ± 1.47	45.10 ± 1.62
	G_3	40.53 ± 1.54	42.15 ± 1.55	43.10 ± 1.51	46.21 ± 1.01
	G_4	39.23 ± 1.80	44.20 ± 1.52	47.21 ± 1.74	50.53 ± 1.43

TABLE 8 Means for the effect of groups and days on the TAC and TOS of rats.

	Group	Days			
		0	15	30	45
TAC (mmol/L)	G_0	2.18 ± 0.02	2.19 ± 0.04	2.17 ± 0.05	2.20 ± 0.07
	G_1	2.03 ± 0.08	2.09 ± 0.05	2.13 ± 0.07	2.14 ± 0.04
	G_2	2.01 ± 0.01	2.76 ± 0.07	2.89 ± 0.01	2.96 ± 0.02
	G_3	2.01 ± 0.09	2.75 ± 0.01	2.83 ± 0.04	2.88 ± 0.05
	G_4	2.02 ± 0.01	2.56 ± 0.09	2.72 ± 0.02	2.87 ± 0.03
TOS (μmol/L)	G_0	7.04 ± 0.12	7.04 ± 0.13	7.07 ± 0.16	7.06 ± 0.15
	G_1	8.40 ± 0.15	8.46 ± 0.19	8.44 ± 0.14	8.43 ± 0.13
	G_2	8.70 ± 0.18	8.56 ± 0.12	7.84 ± 0.17	6.62 ± 0.16
	G_3	8.80 ± 0.15	7.26 ± 0.11	7.16 ± 0.18	6.97 ± 0.14
	G_4	8.36 ± 0.17	7.21 ± 0.13	7.18 ± 0.15	6.67 ± 0.12

sustainable approach can be adopted, minimizing waste generation and generating new functional food products. The findings highlight the potential of PSO and SMPC as effective ingredients for the development of healthier and more sustainable food options. Therefore, it is crucial to further explore and develop these byproducts for the betterment of our health and the environment. Continued research and development in this area will contribute to a healthier and more sustainable future.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by Institutional biosafety committees (IBC) University of Agriculture, Faisalabad.

Author contributions

NI and MAS conceived the work, collected raw materials, carried out experimentations, analyzed and interpreted data, and wrote the article. MRK assisted in experimentations and read the article. MAS, MNF, and MRK supervised the work and review the article. All authors have approved the final article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1199645/full#supplementary-material

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A comprehensive review of summer savory (*Satureja hortensis* L.): promising ingredient for production of functional foods

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This review aims to measure the different aspects of summer savory including biological activity, medicinal properties, nutritional value, food application, prospective health benefits, and its use as an additive in broiler feed. Furthermore, toxicity related to this is also overviewed. Summer savory leaves are abundant in total phenolic compounds (rosmarinic acid and flavonoids) that have a powerful antioxidant impact. Rosmarinic (α-O-caffeoyl-3,4-dihydroxy-phenyl lactic) acid has been identified in summer savory as a main component. According to phytochemical investigations, tannins, volatile oils, sterols, acids, gums, pyrocatechol, phenolic compounds, mucilage, and pyrocatechol are the primary compounds of *Satureja* species. Summer savory extract shows considerable biological potential in antioxidant, cytotoxic, and antibacterial assays. Regarding antioxidant activity, summer savory extract displays an inhibitory effect on lipid peroxidation. Summer savory also has Fe (III) reductive and free radical scavenging properties and contains minerals and vitamins. Summer savory has important biological properties, including antimicrobial activity and antioxidant activity, and protective effects against Jurkat T Cells, Alzheimer's disease, cancer, infection, cardiovascular diseases, diabetes, and cholesterol. The leaves and stems of this plant are employed in the food, feed, and pharmacological industries due to their antioxidant properties and substantial nutritional content. Conclusively, summer savory is widely considered beneficial for human health due to its versatile properties and medicinal use.

KEYWORDS

antimicrobial activity, chemical composition, antioxidant activity, health benefits, nutraceutical, additive, health benefits γ-terpinene, food applications

1 Introduction

Summer savory (*Satureja hortensis* L.) is one of the most popular varieties of savory. It is a seasonal herb that displays similar function and flavor to the perennial winter savory. Autumn savory is used more regularly due to its bitter taste. From July to September in the Northern Hemisphere, this herb blossoms with violet tubular flowers. It has relatively thin brass foliage and rises to a height of 30–60 cm (1–2 ft). *Satureja hortensis* L. is a renowned herb in eastern Canada, and it can be used similarly to sage (Burlando et al., 2010). It is the predominant ingredient in many condiments for fowl, and it is used to produce cretonnade (cretonade). Summer savory is a very rich chicken vinaigrette that can be served with turkey, goose, and duck. It is also used in other food products, including fricot and mince pies. It is frequently accessible in dried form throughout the year in grocery stores, and it is used in varying amounts; for example, it is used in large quantities in cretonnade, whereas it is consumed in smaller quantities in other food products (Brown, 2009). It is popular for seasoning grilled meats and for barbecues, stews, and sauces. Summer savory is recommended for use in sausages over winter savory due to its richer aroma. It is used frequently in Bulgarian dishes, imparting a powerful fragrance in a wide range of meals. The traditional food of Sofia contains three ingredients for seasoning instead of just salt and pepper: salt, crimson chili, and summer savory. Sharena sol is the result of combining these ingredients. In Romanian cuisine, summer savory, also known as "cimbru," is used in "sarmale" (stuffed cabbage and grape leaf rolls) and "mititei" (grilled ground meat rolls) (Cutler et al., 2010). Savory can grow from seeds propagated in a moderately fertile environment. It takes a long time to germinate. Spring season plants are frequently trimmed in June for new usage. The plants can be picked and dried for winter usage when they are in flower (Nybe, 2007). Apart from food preparation, this herb has been used as a traditional antibacterial medicine for gastrointestinal issues (Gelovani et al., 2012). Georgia cultivates native hybrids of summer savory (Akhalkatsi et al., 2012). For instance, Kondari is a variety containing one of the largest total flavonoid concentrations along with the strongest hydro antioxidant activity levels, as discovered in our earlier research on Georgian spices (Rodov et al., 2010). In principle, phenylpropanoid is a precursor of rosmarinic acid, which is the plant kingdom's second leading ester of caffeic acid. Animal investigations have reported that *S. hortensis* powder and its polyphenolic fraction display anti-inflammatory characteristics (Hajhashemi et al., 2002; Uslu et al., 2003). To some extent, this activity has been credited to rosmarinic acid, which has been shown to have anti-inflammatory and anti-allergic effects in human and animal studies (Sanbongi et al., 2003). The antiallergic action of rosmarinic acid has been related to two distinct mechanisms, namely, reactive oxygen species filtration and modification of the inflammatory process (Osakabe et al., 2004). For instance, the nephroprotective impact of rosmarinic acid has been related to an increased antioxidant potency, particularly higher glutathione levels and the antioxidant impact of enzymes (Tavafi and Ahmadvand, 2011).

Summer savory (*Satureja hortensis* L.) also contains a variety of volatile oils (carvacrol and thymol) that have anti-inflammatory (Can Baser, 2008; Hashemipour et al., 2014), antioxidant (Güllüce et al., 2003), antimicrobial (Şahin et al., 2003), and antifungal (Boyraz and Özcan, 2006) properties. Summer savory extract may be valuable to the poultry industry. A previous study reported that dietary summer savory essence (SSE) may enhance and maintain broiler chicken productivity efficiency, blood components, immunological reaction, and ileal microbiota (Movahhedkhah et al., 2019). Furthermore, regarding the volatile oil composition, carvacrol and γ-terpinene are the primary components identified in a typical essential oil of this herb. Therefore, this review comprises different elements relating to summer savory, including an update on the chemical composition of summer savory and its known biological and medicinal properties associated with active substances. Interestingly, specific findings regarding the toxicity of its herb extracts are also provided.

2 Chemical composition of summer savory

Many people in the food industry (herb, vegetable, and fruit growers) see improving human health as a principal goal for this century. Mineral elements are crucial for growth and can support and sustain the human ability to avoid ailments (Grusak, 2002). Herbs are valuable sources of quickly absorbable mineral elements among crops (Boyraz and Özcan, 2006). Summer savory (*Satureja hortensis* L.) is a common herbaceous plant from the Lamiaceae family that is grown in various regions across the world (Jafari et al., 2016) (Kudełka and Kosowska, 2008). Significant levels of minerals (potassium, phosphorus, calcium, magnesium, iron, and sodium) and vitamins (niacin, pyridoxine, riboflavin, thiamine, vitamin A, and vitamin C) were detected in the raw material of *S. hortensis* L. (Mumivand et al., 2010; Karimi et al., 2012; Soltani et al., 2014). Therefore, *S. hortensis* L. may be used as a nutritional basis for essential human minerals. The mineral rank of florae is critical not only for the nutritional content of food but also for the development, growth, and yield of crops. Crucial oils derived from several classes (Khan et al., 2020) in this family possess organic roles including physical function (photosynthesis) and environmental purpose (relationships between the flowers and their environment). Furthermore, the chemical structure of the oils from different *Satureja* strains has been discovered to vary greatly (Slavkovska et al., 2001). Various studies have shown that tannins, volatile oils, sterols, acids, gums, pyrocatechol, phenolic compounds, mucilage, and pyrocatechol are the main compounds of *Satureja* species.

Previous studies have shown the chemical composition of the oils from diverse *Satureja* species varies (Figure 1). The contents of the oils are determined by climatic, periodic, and terrestrial circumstances as well as yield period and purification practice (Baydar et al., 2004). Toward chemical profiling, water has been used to extract crucial oils from air-dried plants and strong spores through 4-h distillation in a Clevenger-type apparatus. Summer savory leaves and seeds had a total of 23 and 24 components, respectively (Farmanpour-Kalalagh et al., 2020). Furthermore, summer savory seeds contain chemicals including Carvacrol, Estragole (Methyl Chavicol), Caryophyllene, and E-Caryophyllene, whereas the leaves are a good source of Carvacrol, γ-Terpinene, and p-Cymene. Moreover, some chemical components are present in both (seeds and leaves), including Carvacrol, Caryophyllene, E-Caryophyllene, β-Bisabolene, cis-α-Bisabolene, Caryophyllene oxide, Z-Citral,

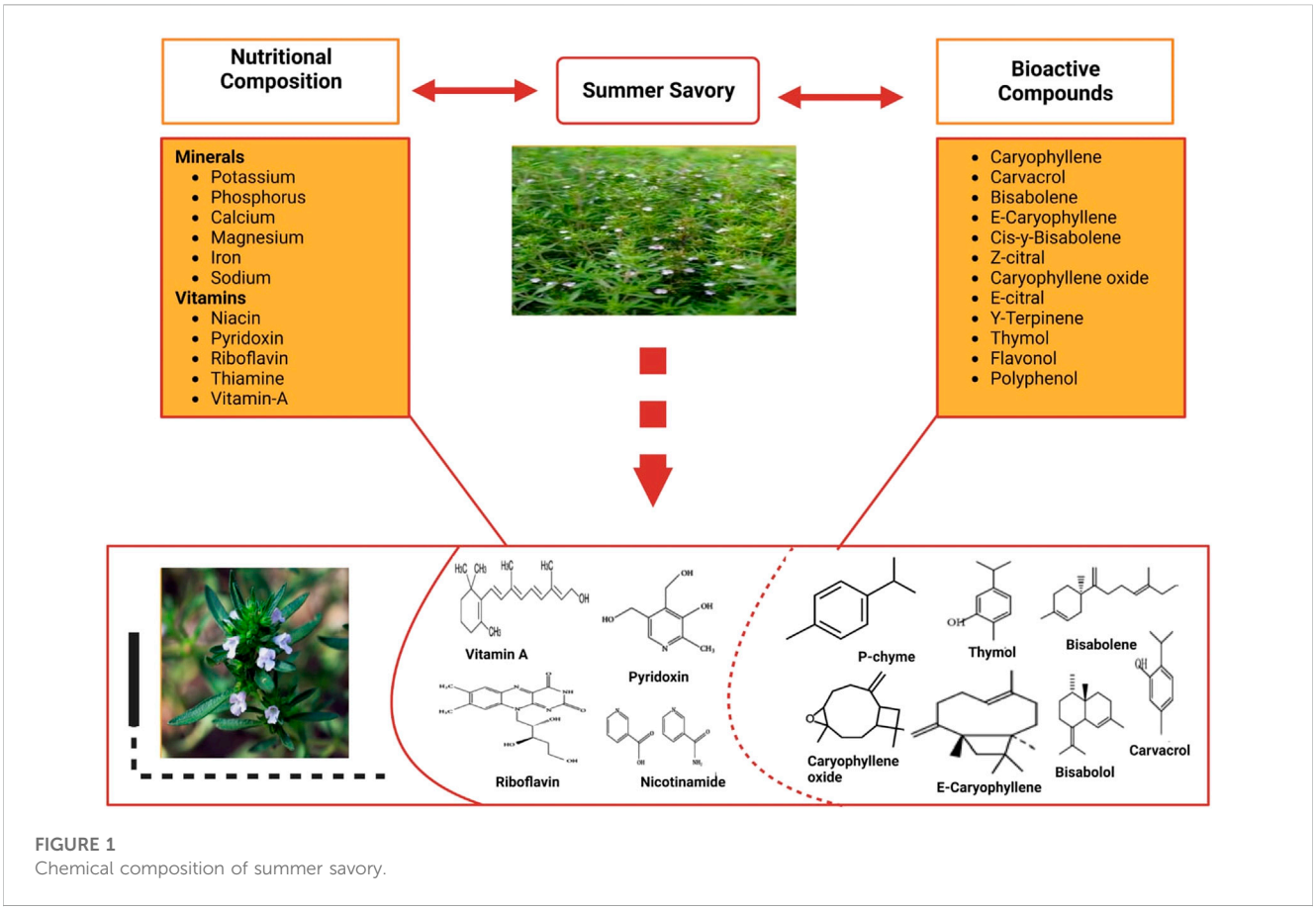

FIGURE 1
Chemical composition of summer savory.

E-Citral, γ-Terpinene, and δ-3-Carene (Farmanpour-Kalalagh et al., 2020). Dried summer savory is composed of volatile oil, which is a vital source of chemicals including carvacrol thymol and monoterpene hydrocarbons (beta-pinene, p-cymene, limonene, and camphene). Vitamins and minerals are present in the leaves of summer savory (Hamidpour et al., 2014). The results of many studies suggest that different parts of summer savory are chemically composed of Estragole, Carvacrol, E-Caryophyllene, Caryophyllene γ-Terpinene, Carvacrol, Thymol and p-Cymene, Caryophyllene, Carvacrol, β-Bisabolene, E-Caryophyllene, cis-α-Bisabolene, Z-Citral, Caryophyllene oxide, E-Citral, γ-Terpinene, and δ-3-Carene (Adiguzel et al., 2007; Kizil et al., 2009; Mahboubi and Kazempour, 2011; Katar et al., 2017; Mohtashami et al., 2018) (Figure 1).

3 Different extraction methods of active compounds from summer savory

The cytotoxic, antioxidant, and bactericidal properties of several *Satureja hortensis* L. extracts have been investigated to perform genetic characterization (Cvetanović et al., 2017). Different separate tests relying on a distinct mechanism have been used in order to evaluate the antioxidant activity of the extracts, including total antioxidant activity, lipid peroxidation inhibition, and hydroxyl radical scavenging (Cvetanović et al., 2017). Phenolic content, including condensed tannins, anthocyanins, and gallotannins, is determined in extracts that are associated with biological activities. Flavonoids are extracted by traditional extraction methods, while rutin is predominantly extracted using unconventional methods (Brighente et al., 2007; Fatima et al., 2023). The previously mentioned bioactive molecules can be separated in different ways (Table 1), i.e., via conventional methods (maceration, solvent extraction, soxhlet extraction, and vapor or hydrodistillation) (Waliat et al., 2023) and innovative technologies (emulsion liquid membrane, ultrasound-assisted extraction, enzyme-associated extraction, pulsed electric field, microwave-assisted extraction, and supercritical fluid) (Aadil et al., 2015; Maqbool et al., 2022; Arshad et al., 2023). A previous study was conducted on the extraction of the chemical composition and biological potential of summer savory extracts using conventional and nonconventional methods. The results verified the domination of the subcritical water approach for the isolation of natural compounds, followed by microwave-assisted extraction (Mašković et al., 2017). Another study was conducted on the extraction of essential oils from summer savory extract, whereby Two extraction methods were compared, namely, microwave-assisted hydrodistillation (MAHD) and traditional hydrodistillation (HD) methods. The outcomes confirmed that the novel method is more suitable compared to the traditional method (Rezvanpanah et al., 2011). The current study of Šeregelj et al. (2022) evaluates the biological activities of ultrasound- and microwave-assisted extracts of S. kitaibelii. The findings confirm

TABLE 1 Extraction of chemical compounds from Summer Savory.

Part of summer savory	Extraction method	Bioactive compounds	Solvent	Reference
Oil (Ariel part)	Conventional	Carvacrol, a-pinene, p-cymene, c-terpinene, and thymol methyl ether	Water/steam	Silva et al. (2005), Skočibušić et al. (2006)
Leaves, flower buds, and calyx	UV-visible spectrophotometry	Flavonoids	Ethanol	Khlebnikova et al. (2022)
Ariel part	Mass spectrometer	Rosmarinic acid, caffeic acid and naringenin acid	Methanol	Boroja et al. (2018)
Flowers, leaves, and steam	Conventional	Phenolic and flavonoids	Ethanol	Predescu et al. (2020)
Flowers	Non-conventional	Rutin and quercetin	Ethanol	Mašković et al. (2017)
Leaves	—	Carvacrol and γ-terpinene	—	Radácsi et al. (2016)
Seeds	Conventional	Carvacrol, c-terpinene, para-cymene; and the minor components a-terpinene, myrcene, camphene, and a-pinene	—	Svoboda and Greenaway (2003)
	GC and GCMS			
Blooming and shade-dried plants	HPTLC and HPLC	Rosmarinic acid (RA), caffeic acid (CA), chlorogenic acid (ChA), apigenin, luteolin, catechin, quercetin, rutin, and hyperoside	Methanol	Shanaida et al. (2021)
Dried	NMR	Luteolin, apigenin, and quercetin	Ethanol and acetone	Exarchou et al. (2002)
Oil (flowers)	GC-MC	α-Phellandrene and myrcene	Helium	Stankov et al. (2022)
Oil	GC-MC	Limonene	Helium	Stankov et al. (2022)
Extract (Ariel parts)	UHPLC/DAD/HESI-MS/MS	hydroxycinnamic acids, caffeic and isoferulic acids	Acetic acid with water	Boroja et al. (2018)
Ariel parts	UHPLC/DAD/HESI-MS/MS	Flavonol (quercetin), flavonol glycosides (isoquercitrin, astragalin, quercitrin), and coumarin derivatives (aesculin and aesculetin)	Acetic acid with water	Boroja et al. (2018)
Dried	HPLC-DAD	Protocatechuic acid	Water and formic acid	Mašković et al. (2017)
Dried	HPLC-DAD	p-Hydroxybenzoic acid	Water and formic acid	Mašković et al. (2017)

that microwave-assisted extraction with water solvent is a promising approach (Šeregelj et al., 2022).

4 An overview of the biological activities of summer savory extracts

Different extracts showed considerable biological potential in antioxidant, cytotoxic, and antibacterial assays (Exarchou et al., 2002). Specifically, the extracts obtained by subcritical water extraction extracts displayed the highest yield. In terms of antioxidant activity, the extract is found to have an inhibitory effect on lipid peroxidation. All manufactured extracts have biological properties, which opens up an extensive variety of potential claims in the nutrition and medicinal industries. Potentially, the extracts can be utilized as normal causes of antioxidants in place of synthetic substances for food preservation as well as the production of functional foods (Güllüce et al., 2003; Şahin et al., 2003).

The medicinal properties of summer savory are shown in Table 2.

Since most of the compounds found in the herb display specific biological properties (Figure 2), these are presented in the following subsections.

4.1 Antimicrobial activity of summer savory

There has been a slew of research in recent years focusing on the antibacterial properties of fragrant florae essential oils and their possible relevance in nutrition protection (Ghalfi et al.). Many studies have been published suggesting a relationship between the chemical construction of essential oil mechanisms and antibacterial properties. In particular, essential oils affect the cell membrane by interacting with and disrupting the phospholipid bilayer and by affecting enzyme activity and genetic resources in bacteria (Rezvanpanah et al., 2011). Essential oil of *S. hortensis* is rich in carvacrol and thymol, which are isomeric composites with a phenylic acid group in their structure. Both thymol and carvacrol suppress the diversity of microbes including bacteria and fungus (Adiguzel et al., 2007; Razzaghi-Abyaneh et al., 2008). The *Satureja* family contains phenolic compounds and their metabolites, which alter the permeability of the cell crust while inhibiting cell respiration. In this way, the *Satureja* family performs antibacterial activity. (Gursoy et al., 2009; Mahboubi and Kazempour, 2011). The antiseptic properties of various herbs and spices have long been known, and they have been utilized in food preservation and healing. (Omidbeygi et al., 2007). Apart from deterioration in foods, fungus development leads to undesired

TABLE 2 Medicinal uses of Summer Savory.

Disease type	Study design	Symptoms	Mechanism	Reference
Alzheimer's	—	Lack of acetylcholine	Part of the *Satureja* spp. contains phenolic compounds such as flavonoids and flavonoid glycosides, which are sources of antioxidants able to diminish the development and evolution of Alzheimer's disease and decrease neuronal degeneration	Öztürk (2012)
Inflammatory bowel disease	Mice	Inflammation	Phenolic acids help to reduce inflammation	Hajhashemi et al. (2002), Rocha et al. (2007)
			The polyphenols and essential oil of *Satureja* spp. exhibit significant anti-inflammatory activity. The literature reports the traditional employment of *S. hortensis* as a solution for inflammation diminishing and pain relief	
Cancer	Humans	Lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements	The effect of carvacrol on a human non-small cell lung cancer (NSCLC) cell line also named A549 demonstrated the inhibitory action of carvacrol on cancer cells. The research shows that carvacrol could present anti-carcinogenic activity and could be employed in cancer treatment	Koparal and Zeytinoğlu (2003), Kennedy et al. (2018)
Rhino-sinusitis	Rabbit	nasal discharge, sneezing, and swelling of the nose	The anti-inflammatory activity of *Satureja hortensis* L. was investigated by evaluating NO● metabolites. The results confirmed the potential of *Satureja hortensis* L. extract in inflammation reduction, thus suggesting its use in the treatment of rhino-sinusitis diseases	Uslu et al. (2003)
Heart-related	Humans	Difficulty in breathing, heartburn, nausea, vomiting, abdominal pain, and cold sweats	Literature states that extract of *S. hortensis* L. in methanol reduces blood platelet adhesion, aggregation, and secretion, thus explaining its traditional employment in cardiovascular and blood clot disease treatment	Mihajilov-Krstev et al. (2010)
Diabetes	Humans	Weight loss	Polyphenols are considered great natural antioxidants that have been demonstrated to act similarly to anti-diabetic medicines, which decrease the glucose concentration of blood	Ahmadvand et al. (2012), Ramachandran (2014)
		Frequent fatigue		
		Dry mouth		
		Burning and pain in feet		
		Itching		
		Decreased vision		
Hepatitis B	Humans		The antiviral properties of Savory spp. essential oil were investigated, and some results revealed that *Satureja boliviana* can slow down the actions of hepatitis B, herpes simplex type 1 virus (HSV-1), and vesicular stomatitis virus	Bezić et al. (2009)
				Momtaz and Abdollahi (2010)
Genotoxin	Rats	Oxidative stress	SHE (*S. hortensis* ethanolic extract) displayed a considerable inhibitory effect on oxidative DNA damage	Behravan et al. (2006)
			SHEO (*S. hortensis* essential oils) also displayed appreciable inhibitory activity on H_2O_2 induced chromosomal damage	
Antifungal	—	—	The essential oils extracted from plants have many advantages compared to traditional chemical fungicides, which makes their future use promising	Shirzad et al. (2011)
Antioxidant, Hepato-protective	Rats	Oxidative stress	A single dose of cisplatin (7.5 mg/kg) produced damage in the liver, as demonstrated by the rise in serum ALT, ALP, AST, and GGT contents. Adjuvant treatment with *S. hortensis* extract generated a considerable reduction in serum AST, ALT, and ALP quantities, demonstrating its hepatoprotective activity	Boroja et al. (2018)

(Continued on following page)

TABLE 2 (*Continued*) Medicinal uses of Summer Savory.

Disease type	Study design	Symptoms	Mechanism	Reference
Antinociceptive, Anti-inflammatory	Male mice	Inflammation	Decreased acetic acid-induced abdominal twitches. Hydroalcoholic extracts considerably lowered the pain responses in the early and late phases of the formalin test, while the polyphenolic extract and essential oil demonstrated effectiveness only in the late phase of the formalin test	Hajhashemi et al. (2012)
Antioxidant, Cytotoxic, Antibacterial	—	—	The extracts displayed antioxidant, cytotoxic, and antimicrobial activities, with the greatest biological potential exhibited in the case of subcritical water extracts	Mašković et al. (2017)
Antimutagenic	Humans	—	Phenylpropanoids and phenolic molecules like flavonoids, phenolic acids, and phenolic monoterpenes were proven to be responsible for antimutagenic activity in aromatic plants	Caillet et al. (2011)
Protective effect against AFB_1 mutagen, Antioxidant	Humans	Increased MN frequencies, oxidative stress	Luteolin was demonstrated to possess many health benefits. Research regarding luteolin derivatives could contribute to the knowledge of their positive effects	Orhan et al. (2016)

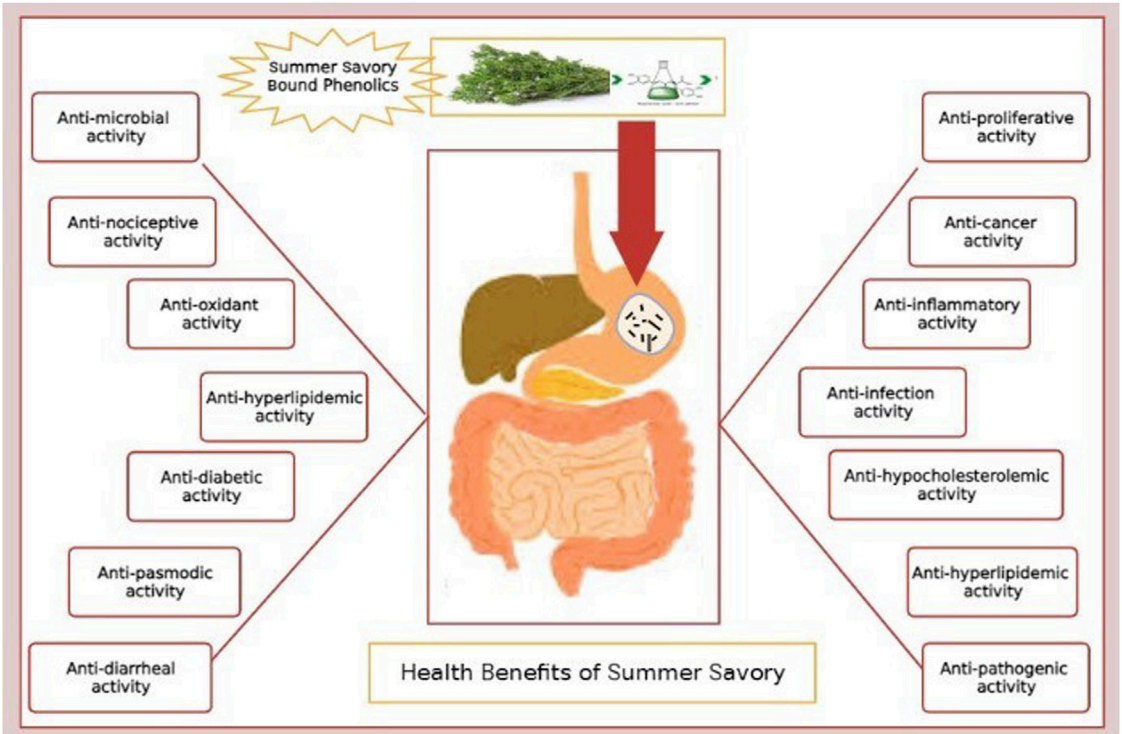

FIGURE 2
Prospective health benefits of summer savory.

metabolites such as aflatoxin, which may be derived from Aspergillus species (A. parasiticus and A. flavus). Additionally, mycotoxin (toxigenic fungus) can be found in food and grains that have been preserved for a long time. In both animals and humans, aflatoxins are known to be strong hepatocarcinogens. To some extent, antifungal properties have been discovered in several savory species (Dikbas et al., 2008). Summer savory ingredients can be used as an additional preservative in food items due to their antibacterial properties (Rezvanpanah et al., 2011). The essential oil from some savory species has been shown to be high in antiviral properties (Bezić et al., 2009). The seeds and leaves of summer savory are good sources of essential oil. The essential oil of summer savory is composed of chemical compounds (hydrophobic and hydrophilic molecules) that play an important role in

antimicrobial activity. The hydrophilic and hydrophobic molecules play a favorable role in antimicrobial activity. The outer layer of Gram-positive bacteria is a peptidoglycan cell wall that allows hydrophobic molecules to penetrate and reach the internal material, whereas the outer layer of Gram-negative bacteria is lipopolysaccharide, which allows mainly small hydrophilic molecules to pass and is only partly permissive for hydrophobic molecules. The hydrophobicity of essential oils is responsible for the disruption of microbial structures. The essential oil has different mechanisms of action on the microbial population, including degradation of the cell wall and cytoplasmic membrane, cytoplasm coagulation, and diffusion through the double lipid layer of the membrane, together with alteration of its permeability and function (Nazzaro et al., 2013). *In vitro* investigations have shown that the *Satureja boliviana* could suppress the overall effects of vesicular stomatitis virus (VSV), hepatitis B, and herpes simplex type 1 virus (HSV-1). However, S. montana can protect against HIV-1 virus (Omidbeygi et al., 2007). It has been discovered that *S. hortensis* essential oil has excellent antifungal action compared to *Aspergillus flavus.* Moreover, it could be exploited as a cause of environmental plant antifungals to protect various food products from infection and saprophytic fungi. *S. hortensis* essential oil has a broad antibacterial spectrum that inhibits the progress of the social and phytopathogenic bacteria, fungi, and yeasts that cause food spoiling. Vital oils from *S. hortensis* were found to be effective against *S. aureus, Listeria monocytogenes, S. typhimurium,* and *E. coli* O157:H7 along with the *Pseudomonas putida* strain obtained from meat (Oussalah et al., 2006). Essential oils from summer savory reduce the mycelial development of plant-pathogenic fungi (*Botrytis cinerea* and *Alternaria mali*) due to their antifungal properties (Boyraz and Özcan, 2006). Furthermore, the oil was shown to reverse the progress of aflatoxin (AFG1 and AFB1) by *Aspergillus parasiticus in vitro* under storage conditions (Dikbas et al., 2008), in liquid standard and in tomato adhesive (Omidbeygi et al., 2007), and reverse the development and production of aflatoxin (AFG1 and AFB1) by *A. parasiticus* (Razzaghi-Abyaneh et al., 2008). The antiseptic effect of *S. hortensis* essential oil was tested and compared to selected strains using the broth micro-well dilution method. Vital oil was found to be effective against entirely medical insulates from injuries that were verified. The oil has the best antibacterial action against *Acinetobacter spp.* and *S. aureus* (Pintore et al., 2002). It also displays activity against *Staphylococcus* spp. and E. *coli* (Wilkinson et al., 2003). In identical concentrations, the oil remains efficacious against *Enterobacter spp.* and *Enterococcus* spp., *S. pyogenes, P. mirabilis*, (Wilkinson et al., 2003). The oil's significant antibacterial activity is due to its high concentration of phenol component carvacrol, which has already been proven to have antimicrobial activity (Ben Arfa et al., 2006). Carvacrol has been found to have substantially strong antibacterial potential compared to other chemically similar compounds, such as eugenol (Ben Arfa et al., 2006).

4.2 Antioxidant activity of summer savory

Antioxidants are substances that prevent the oxidation of other compounds by preventing or delaying the beginning or proliferation of oxidative chain reactions (Roobab et al., 2018; Mukhtar et al., 2023). Fears about the antagonistic properties of artificial antioxidants have recently prompted the consumption of natural antioxidants found in all plants and all their parts (Bakkali et al., 2008). Numerous studies have proven that *Satureja* strains display antioxidant action (Exarchou et al., 2002). Previous studies have stated that the oils of *Satureja* species are a rich source of isopropanoids and flavonoids, such as p-cymene, linalool, carvacrol, thymol, β-caryophyllene, and γ-terpinene. These compounds have powerful antioxidant properties (Ruberto and Baratta, 2000). It has been determined that the antioxidant consequence of fragrant plants may be related to the existence of hydroxyl groups in their phenolic chemicals. In *S. montana,* components with hydroxyl groups have relatively significant antioxidant activity (Radonic and Milos, 2003). During storage, the *Satureja cilicica* essential oil showed substantial antioxidant activity in butter. Moreover, with increased concentrations of oil, the antioxidant properties of rose oil also increased (Ozkan et al., 2007). The outcomes of the study of Ozkan et al. (2007) show that the essential oil of *S. cilicica* can be employed as a natural antioxidant and fragrance agent in fat. Oxidative damage is induced by the formation of sensitive oxygen species (SOS) throughout ordinary cell aerophilic respiration, and it plays a key part in the start and progression of several illnesses in the human body. Antioxidants play a significant role in defending cells from oxidative compensations and in the prevention of a variety of diseases (Szőllősi and Varga, 2002). This study suggests that the methanolic extract of the S. hortensis aerial part may be valuable against cisplatin-induced oxidative damage in the liver, kidney, and testes of rats (Boroja et al., 2018). Another study verified that S. hortensis extract is a good source of antioxidants. However, different methods were used to measure antioxidant properties. The outcomes of FRAP, ABTS, and DPPH measured the high activity of the S. hortensis extract. Moreover, the total phenolic and total flavonoid content was also determined as high in S. hortensis extract (Mašković et al., 2017). Previous studies have proven that leaves and essential oil are important sources of chemical compounds including isopropanoids, rosmarinic acid, and flavonoids. The outcomes confirm that both leaves and oils have a high antioxidant capacity (Momtaz and Abdollahi, 2010; Chkhikvishvili et al., 2013).

4.2.1 Summer savory protects Jurkat T Cells against oxidative stress

Rosmarinic acid is the main phenylpropanoid component in summer savory. Jurkat cells can be protected against oxidative stress generated by hydrogen peroxide by *S. hortensis* and its rich rosmarinic acid proportions. The results of Hajhashemi et al. (2002), Sanbongi et al. (2003), Osakabe et al. (2004) remain consistent through the cytoprotective, antiinflammatory, and antioxidant activities of *S. hortensis* (Hajhashemi et al., 2002) and rosmarinic acid (Sanbongi et al., 2003; Osakabe et al., 2004) in animals and humans. *S. hortensis* extracts exhibited significant protective antioxidant actions when administered to H_2O_2-stressed lymphocytes isolated from healthy rats' blood (Behravan et al., 2006). Rosmarinic acid protected human neuronic cells from hydrogen peroxide-induced apoptosis in cell cultures (Lee et al., 2008) and inhibited the creation of reactive nitrogen and oxygen species in RAW264.7 macrophages encouraged with phorbol 12-myristate 13-acetate or lipopolysaccharides in a dose-dependent

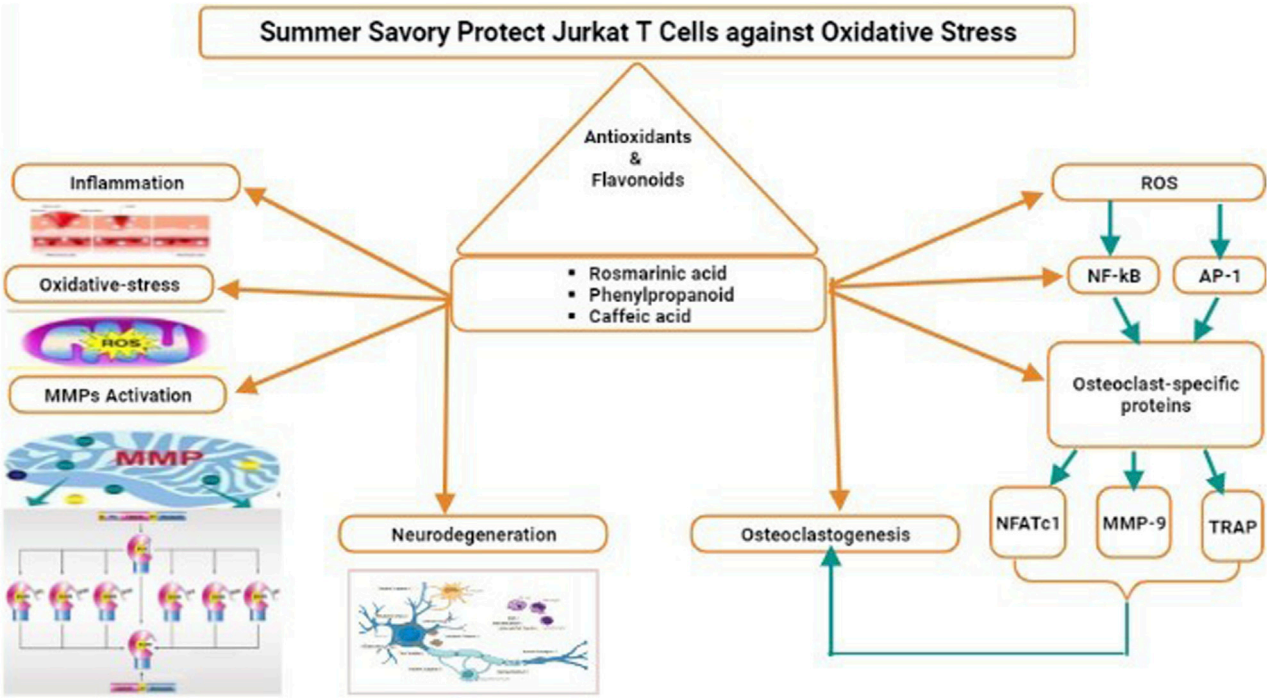

FIGURE 3
Potential role of summer savory in Jurkat T cell against oxidative stress.

manner. However, maximum phenolic composites demonstrated pro-oxidant properties at small dosages in a metal catalyst system before switching to antioxidant activity at higher concentrations (Fukumoto and Mazza, 2000). Furthermore, high dosages (2–3-mM) of caffeic acid and phenylpropanoids have recently been demonstrated to protect Jurkat cells from H_2O_2-induced DNA impairment by chelating intracellular labile iron (Kitsati et al., 2012). In addition to rosmarinic acid, the existence of powerful antioxidants in the phenolic element may enhance its antioxidant potential. *S. hortensis* may also aid in the neutralization of hydrogen peroxide by increasing the activity of antioxidant enzymes. In Jurkat cells, SOD and Catalase play crucial roles in the regulation of apoptosis and oxidative stress (Kagan et al., 2002). The extract of *Perilla frutescens* rosmarinic acid in water from a Lamiaceae plant was demonstrated to have effects on the protein and mRNA appearance of the antioxidant enzymes in cultivated human vein endothelial cells (Saita et al., 2012). Anti-inflammatory features like IL-10 may be generated in stressful settings to counteract the rapid rise in pro-inflammatory cytokines and regulate the amount and interval of the inflammatory response. The accumulation of antioxidant-rich herbal ingredients in the diet of wildlife suffering from pro-inflammatory conditions has been demonstrated to enhance IL-10 levels (Kim et al., 2011) or both IL-2 and IL-10 (Zhang et al., 2012) in tandem, with a decrease in pro-inflammatory markers like IL-6, TNF-α, and IL-1β levels. Furthermore, nutritional interferences conserved standard antioxidant enzyme action, prevented fat peroxidation, and boosted HDL levels in the preserved animals, leading to improved immunity and the alleviation of diseases (Zhang et al., 2012). In a lipopolysaccharide-stimulated macrophage model, rosmarinic acid boosted 1L-10 productions (Mueller et al., 2010). Adding the *S. hortensis* extract or its phenolic component to H_2O_2-challenged Jurkat cells restored survival and proliferation, relieved the G0/G1 arrest, and regulated apoptosis. Overall, these findings are consistent with the overall reaction of cells to oxidative stress, whereby small quantities of reactive oxygen species encourage cell growth, in-between measures cause evolution arrest, and high oxidative stress causes cell death through necrotic or apoptotic mechanisms (Martindale and Holbrook, 2002). The accumulation of *S. hortensis* extracts appears to reduce the oxidative stress caused by hydrogen peroxide on the cells. These effects may be related to phenolic compounds and rosmarinic acid direct radical-scavenging activity but also to unintended processes like the increase in antioxidant enzymes and the production of anti-inflammatory gesturing particles like IL-10 (Figure 3).

4.2.2 Fe (III) reductive and free radical-scavenging properties of summer savory

The capacity of complexes and herb extracts to decrease Fe (III) is frequently employed as a measure of electron contributing procedure. This may be due to the phenolic antioxidant mechanism (Yildirim et al., 2000). The components of Summer Savory are resolvable in acidic aqueous methanol solvent and they have the ability to donate electrons to the unbalanced free radicals (Dorman et al., 2003). These are further constant non-reactive classes, with the EtOAc-soluble mechanisms acting as maximum active electron donors (Dorman et al., 2003). ABTS and DPPH artificial free radicals and hydroxyl radicals are used to examine the possible free radical-scavenging actions of the *Satureja hortensis* L. extract and subfractions (Jayasinghe et al., 2003). ABTS and DPPH

are widely employed to estimate free radical-scavenging capabilities (Jayasinghe et al., 2003). There are often problems with solubility and interference with DPPH tests; thus, ABTS free radicals are frequently utilized (Arnao, 2000). However, it has been suggested that these approaches are not able to use a biologically or food-related reactive species and oxidizable substrate. Moreover, they can only indicate potential antioxidant activity and, therefore, provide no direct information on defensive presentation (Güllüce et al., 2003). Phospholipids are thought to play a key role in off-flavor development and oxidative deterioration in foodstuffs (Frankel and Meyer, 2000). The capability of the models to prevent ascorbate-Fe (III)-generated hydroxyl radical-mediated peroxidation of a heterogenous phospholipid-aqueous phosphate buffered system is resolute, and the hydroxyl radical is an extremely volatile radical that is used in in vivo study. *In vitro* studies have shown that the EtOAc-soluble mechanisms are activated at the maximum level, including crude extract and Fe (III) decrease assay. With hexane, EtOAc, and n-BuOH, an aliquot part of this extract was sub-fractioned by liquid-liquid breakdown against water (Kim et al., 2003). Fe (III) reduction assays as well as ABTS, DPPH, and hydroxyl free radical-scavenging assays were used to characterize these materials' antioxidant capabilities (Hajhashemi et al., 2000). The EtOAc fraction and crude extract were the maximum active samples, showing much lower activity (Antolovich et al., 2002). The crude extract containing EtOAc-soluble components had predominantly significant action when used as preservative to prevent free radical-mediated destruction of vulnerable components. Moreover, free radicals play a key role in the deterioration of human health. The EtOAc subfraction may have favorable impacts on human biology when it is used in adequate amounts in foods (Niki et al.).

4.3 Alzheimer's disease

Alzheimer's disease is generally caused by a deficiency of acetylcholine, which is a neurotransmitter. Alzheimer's disease has been treated using acetylcholinesterase inhibitor tablets. Nevertheless, these treatments may involve adverse side effects. To prevent this sort of disease, the production of natural chemicals with antioxidant and anticholinesterase properties is preferable (Öztürk, 2012). Antioxidants can play a significant role as neuroprotective agents at the initial stage of Alzheimer's disease (Silva et al., 2009). Some *Satureja* species are vital sources of phenolic compounds (flavonoids and flavonoid glycosides) that can play an important role as antioxidants. However, the antioxidant potential of *Satureja* species may reduce the chances of developing Alzheimer's disease and neuronal degeneration (Öztürk, 2012). The activation of cell signaling pathways occurs due to oxidative stress. The results of Pritam et al. (2022) show reduced formations of toxic substances that foster the development of the disease. Antioxidants reduce free-radical-mediated damage in neuronal cells through detoxification. Moreover, the balance between antioxidants/oxidants is unfavorably unbalanced, which can have detrimental effects, such as Alzheimer's disease (Sinyor et al., 2020). One study shows that the phenolic content of several *Satureja* species, specifically flavonoids and flavonoid glycosides, which delay the growth of Alzheimer's disease and lower neural degeneration due to potent antioxidants (Öztürk, 2012). Thymol and carvacrol are abundant in *Satureja* species that can work as low cholinesterase inhibitors and protect people from oxidative stress and amnesia despite causing no adverse side effects (Öztürk, 2012). The study of Ross et al. (1999) shows that the progression of Alzheimer's disease can be reduced by reducing neuronal damage. Previous studies have verified that different savory species can protect against various chronic diseases, including Alzheimer's, diabetes, cancer, and cardiovascular diseases (Hamidpour et al., 2014).

4.4 Cancer

The plant materials are composed of phenolic chemicals that can help to protect or reduce oxidative destruction by both non-free-radical and free-radical species (Alizadeh et al., 2010; Ali et al., 2022). Regulation of oxidative chain reaction formation and growth helps to prevent various disorders, including oxidative stress dysfunctions, cancer, heart disease, and neural disorders (Szőllősi and Varga, 2002; Alizadeh et al., 2010). The anti-carcinogenic, vasoprotective, anti-allergic, anti-proliferative, anti-inflammatory, and antimicrobial properties of phenolic acids and flavonoids have been documented in several studies. *Satureja montana* L. has been used as medication in the treatment of several types of cancer (Cetojevic-Simin et al., 2008). The extracts suppressed the development of HT-29 (human colon adenocarcinoma) cells at levels over 0.7 mg/mL, and HeLa (human cervix epidermoid carcinoma) was shown to present the maximum sensitivity to the savory extract. Several *Satureja* spp. species are powerful sources of antioxidants that can prevent the growth of an extensive variety of human tumor cells (Cetojevic-Simin et al., 2008). Food products have been demonstrated to be effective and stable sources of innovative medicine. Carvacrol is a monoterpene present in the essential oils of a diversity of fragrant plants. The activity of carvacrol on the human non-small cell lung cancer (NSCLC) cell line named A549 indicates that it reduces cancer cells but has no impact on normal lung cells (HFL1). The findings of Koparal and Zeytinoğlu (2003) suggest that carvacrol has anti-carcinogenic properties and can be used as a cancer treatment medicine.

4.5 Anti-infection properties

Bacteria, fungi, and viruses are the most common sources of illnesses that affect both flora and fauna. Plant essential oils act as secondary metabolites to protect humans from natural enemies. It may be genetic or in reaction to pathogens. The antimicrobial activity of *Satureja* spp. was initially discovered in the 1950s, and it was discovered that the inhibitory action of savory is probably due to its high carvacrol and thymol content. These are the two most effective herbal antiseptic compounds (Oussalah et al., 2006). Essential oil concentration and composition are influenced by storage circumstances as well as the concentration and type of the mark microorganism (Baydar et al., 2004). Essential oil concentrations in *Satureja parnassica* and *Satureja thymbra* have been found to fluctuate. The oils extracted during the flowering time were have been determined as the most potent, with minimum inhibitory concentration (MIC) standards and significant antibacterial activities (Chorianopoulos et al., 2006). Essential oils have been shown to have inhibitory effects against a diverse variety

of food-deteriorating bacteria due to their concentration in valuable components (Skočibušić et al., 2006). Furthermore, different varieties of *Satureja* have also been widely studied for their resistance to foodborne diseases. In Greece, the essential oils extracted from *Satureja* spp. contain monoterpene hydrocarbons and phenolic monoterpene. These oils have outstanding antibacterial properties against foodborne pathogens (Chorianopoulos et al., 2004). Summer savory (*S. hortensis* L.) extract was investigated in relation to the mycelial growth of food fungi due to the fungicidal activity of the hydrosol (Boyraz and Özcan, 2006). The extract was found to have dose-dependent fungicidal activity at all dosages. Carvacrol has been shown to be most effective in *S. thymbra*, followed by p-cymene and monoterpene hydrocarbons c-terpinene (Soković et al., 2002). The effect of *S. boliviana* against binary distinct VSV and viruses-HSV-1 has also been investigated. The active component in the aqueous extract of *S. montana* has been discovered to be non-polar water-soluble molecules. Non-polar chemicals like essential oils and extracts have substantial anti-HIV-1 action (Abad et al., 1999).

4.6 Cardiovascular diseases

Cardiovascular issues and thrombosis (blood accumulations in the vein or artery) occur due to platelet hyperactivity that contribute to their adsorption to the vascular wall (Yazdanparast and Shahriyary, 2008). Cardiovascular diseases (CVD) are usually illnesses that are directly related to blood arteries and the heart. Excessive oxidative stress produces reactive oxygen species (ROS) that are responsible for the pathophysiology of different CVDs such as ventricular remodeling, cardiomyopathy, myocardial infarction, heart failure, cardiac hypertrophy, and atherosclerosis. The body's endogenous system fails to maintain normal physiology due to excessive oxidative stress. However, different sources of antioxidants are necessary to scavenge free radicals (Jain and K Mehra N, 2015). Different investigations have shown that *S. hortensis* has anticoagulant blood properties. Flavonoid, monoterpene hydrocarbons, and carvacrol, including phenolic acids and apigenin (labiatic acid), may all play a role in *S. hortensis* anti-platelet activity (Yazdanparast and Shahriyary, 2008). The methanol extract of *S. hortensis* has been shown to reduce secretion, aggregation, and blood platelet adhesion, which may explain its conventional usage in the treatment of blood clots and cardiovascular issues (Mihajilov-Krstev et al., 2010). The current study of Khalid et al. (2023) suggests that essential oil, antioxidants, and phenolic compounds protect CVDs by reducing cholesterol, preventing oxidation, and reducing platelet aggregation, respectively. Previous studies have suggested that phenolic compounds reduce the risk of CVDs by preventing atherothrombosis and platelet activity (Torres-Urrutia et al., 2011; Fuentes et al., 2012).

4.7 Antidiabetic and anticholesterol effects

Hypertension and hyperlipidemic raise the threat of cardiac ailment. It has been recognized that hypercholesterolemia has been linked to a variety of clinical conditions including atherosclerosis, diabetes mellitus, thromboembolic, and cardiovascular diseases. Antioxidants play a vital role in the handling of illnesses involving oxidative stress damage (diabetes mellitus) (Vosough-Ghanbari et al., 2010). Antioxidant medication is the best way to prevent and decrease the development of diabetic consequences, including hyperlipidemia and liver issues. Natural antioxidants derived from medicinal herbs have recently captivated researchers' curiosity as a possible replacement for artificial antioxidants (Ahmadvand et al., 2012). Polyphenols are well-recognized natural antioxidants that have been shown to reduce blood glucose levels in a manner comparable to antidiabetic medications. *Satureja khuzestanica* (SKE) is an Iranian *Satureja* plant with antioxidant activities and anti-diabetic actions (Momtaz and Abdollahi, 2010). Malondialdehyde levels and serum glucose were controlled in diabetic patients using SKE (Momtaz and Abdollahi, 2010). SKE essential oil has been investigated for its hepatoprotective, hypolipidemic, and antiatherogenic properties. In diabetic patients, it can reduce the risk of cardiovascular mortality and liver injury (Ahmadvand et al., 2012). Thymol and carvacrol are the essential components of *Satureja* species. Essential oils have been revealed to reduce serum cholesterol levels. Flavonoids are abundant in *Satureja* species that have been shown to have anti-hyperlipidemic and antioxidant effects. SKE has been shown to significantly reduce the triglyceride levels and fasting blood glucose in hyperlipidemic and diabetic rats along with ATP levels and lipid peroxidation in numerous trials (Momtaz and Abdollahi, 2010). The study of Momtaz and Abdollahi (2010) indicates that some *Satureja* species, including SKE, can be utilized as an enhancement in hyperlipidemic diabetic patients due to their lipid-lowering and antioxidant characteristics (Vosough-Ghanbari et al., 2010). The flavonoids extracted from summer savory reduce cholesterol in rabbits and lead to a considerable decrease in serum cholesterol (Mchedlishvili et al., 2005). Rats ingesting *S. khuzestanica* essential oils showed a considerable increase in total antioxidant capacity and a reduction in normal blood lipid peroxidation levels *S. khuzestanica* oil's antioxidant properties may explain its triglyceride-lowering properties (Abdollahi et al., 2003). Isopropanoids such as carvacrol, thymol, and flavonoids are also important components of *S. khuzestanica*. Thymol and carvacrol have been demonstrated to lower serum cholesterol stages considerably. Flavonoids have also been revealed to have anti-hyperlipidemic effects (Momtaz and Abdollahi, 2008).

4.8 Anti-inflammatory and analgesic effects

Inflammation is the body's natural defense process in relation to pathophysiological conditions. Several examinations have been conducted in order to discover extra influential anti-inflammatory treatments with less harmful properties (Amanlou et al., 2005). Plants of the Lamiaceae family are recognized for their pain-relieving and antispasmodic effects. In animal experiments, many components of *Satureja* species (flavonoids) have been found to be essential for analgesic, relaxing, and vasodilatory actions (Momtaz and Abdollahi, 2010). In addition to analgesic effects, various *Satureja* species have been identified to exhibit anti-inflammatory properties (Momtaz and Abdollahi, 2010). *S. hortensis* L. has been used as a bone pain and muscular reliever in traditional remedies Previous studies have shown that polyphenolic and essential oil from *Satureja* spp. have strong

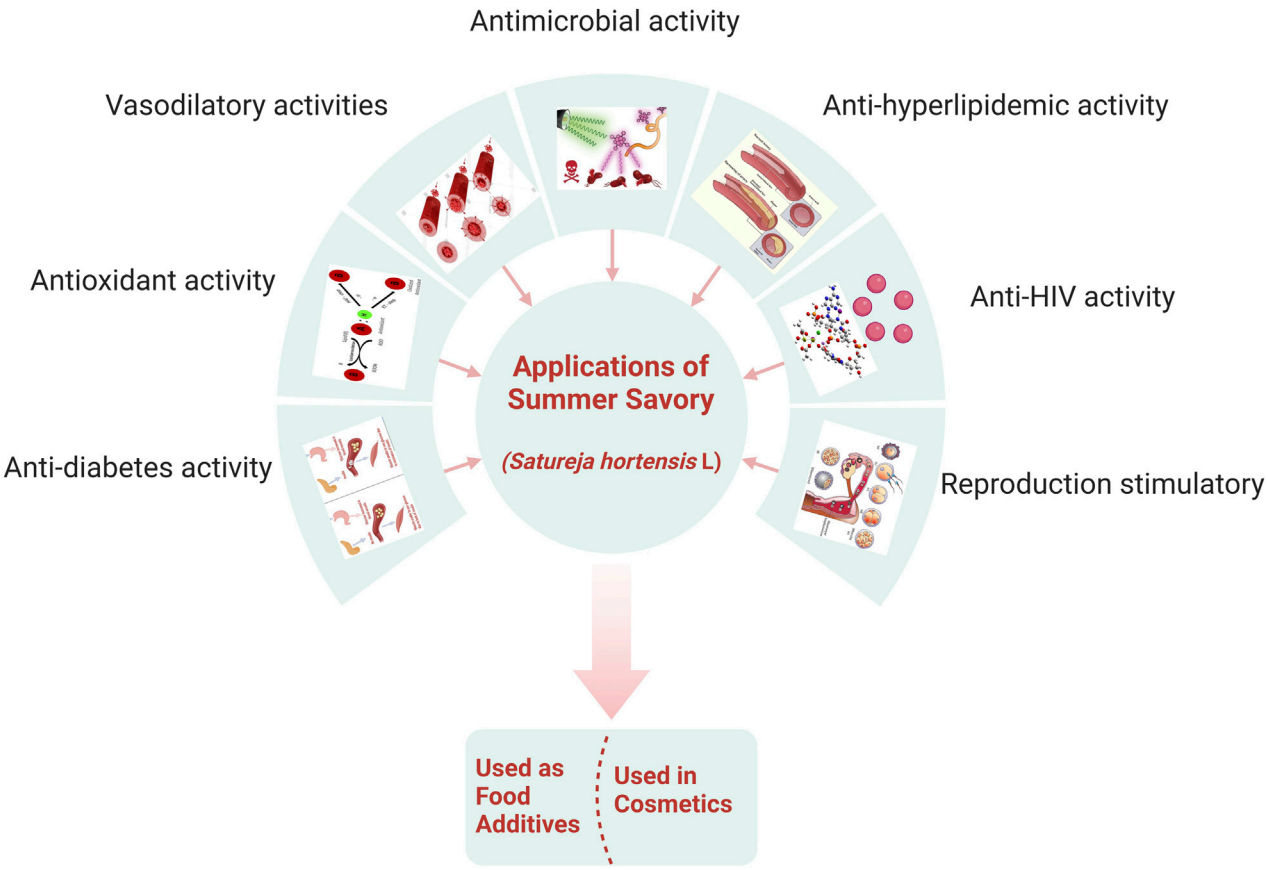

FIGURE 4
Different applications of summer savory.

anti-inflammatory characteristics. The research confirms the traditional use of *S. hortensis* as a pain killer and an anti-inflammatory (Hajhashemi et al., 2002). According to several investigations, *Satureja* species, including *S. hortensis* and SKE, act as anti-inflammatory medications and are equivalent to morphine, indomethacin, and prednisolone (Momtaz and Abdollahi, 2010). Animal and human trials have shown the anti-inflammatory and anti-allergic actions of savory linked to the polyphenolic fraction (rosmarinic acid) (Chkhikvishvili et al., 2013).

4.9 Summer savory and reproduction stimulatory effects

A study was conducted on male rat fertility in which *S. khuzestanica* essential oil significant gains in fertility index, fecundity, litter index, and potency Additionally, it reduced the post-implant loss (Haeri et al., 2006). Moreover, the seminal vesicles, ventral prostate, and weights of the testes were greatly elevated, and the weight of the testes, testosterone, and FSH were concentrated. These alterations could be linked to the antioxidant potential of the essential oils. The principal antioxidants in *Satureja* spp. are pcymene, carvacrol, and flavonoids. The results indicate the stimulatory effects of this genus on reproduction (Radonic and Milos, 2003).

5 Applications of summer savory

S. hortensis is used worldwide as a food additive, flavoring, and spice and in herbal beverages due to its pre-eminent ethnomedical activity and pharmaceutical and food applications (Figure 4). Furthermore, summer savory oil has been used in the cosmetic industry and in perfumes, both alone and in combination with essential oils (Sefidkon et al., 2004).

Primarily, savory species are used to treat muscle pain, flatus, and intestinal disorders such as indigestion, cramps, nausea, and diarrhea (Abdollahi et al., 2003). Other properties of *S. hortensis* include antifungal, antibacterial, antioxidant, anti-hyperlipidemic, anti-HIV, anti-diabetes, expectorant, reproduction stimulatory, and vasodilatory activities (Şahin et al., 2003; Amanlou et al., 2005; Basiri et al., 2007). In ancient medical books, it is demonstrated that *Satureja* spp. has a medicinal impact on respiratory diseases, such as coughs and asthma (Vagionas et al., 2007).

5.1 Summer savory used as a native additive in broiler feed

Essential oils have been revealed to have various favorable properties in broiler feed, including increased feed intake (Jang

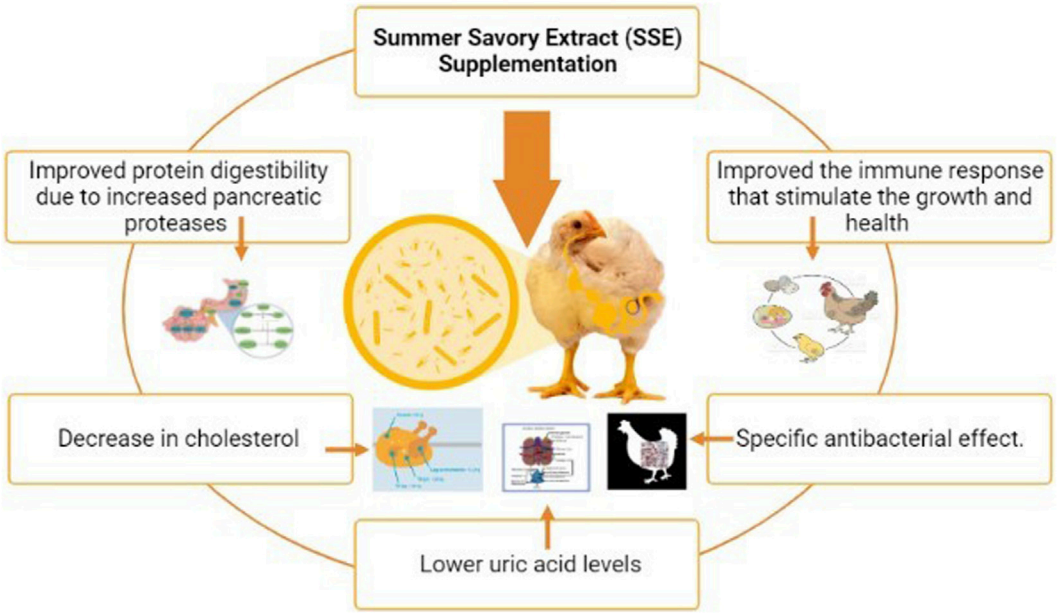

FIGURE 5
Summer savory use as supplementation.

et al., 2007), improved digestibility, higher digestive enzyme secretion (Jamroz et al., 2005), and microbial ecosystem balancing (Liolios et al., 2009). The beneficial qualities of the essential oils of this plant may account for the increased performance reported, with the addition of summer savory essence (SSE). The plasma content of feed could be linked to the effects of the essential oils in summer savory foods on digestion. In broilers, feed supplementation with thymol was found to greatly improve pancreatic action (Lee et al., 2003). Protein digestibility improved due to increased pancreatic proteases, which could explain why the SSE-supplemented sets had lower uric acid levels. The cholesterolemic action of essential oils (Nobakht et al., 2012) could explain the considerable decrease in cholesterol SSE addition in the diet at 200 and 300 ppm, along with LDL at 300 and 400 mg/kg SSE. SSE-supplemented diets improved the immune response of broilers as they grew older; the immune response, being dependent on age, may be attributable to the essential oil content and antioxidants (Attia and Al-Harthi, 2015). The considerable decrease in *E. coli*, correspondence to *Lactobacillus spp*., and a greater ratio of *Lactobacillus* to coliform suggest that SSE-treated birds have better gut health. This array of IB and IBD virus titers, *Lactobacilli* counts, and *E. coli* may indicate that savory extract has a specific antibacterial effect (Attia et al., 2017). Furthermore, better gut biology could explain the rise in antibody titers due to the nutrition sparing-effect (Attia et al., 2017). Although there was no substantial impact of SSE supplementation in broiler feed, conversion ratio and body weight gain were considerably enhanced when 400 mg/kg SSE was used (Bombik et al., 2012). SSE supplementation enhanced the majority of the blood indicators and immunological response criteria evaluated (Bombik et al., 2012). *Lactobacilli* count was unaffected by diet. However, SSE lowered the *Escherichia coli* count and improved the *Lactobacillus* to coliform ratio (Bombik et al., 2012). Supplementing the broiler feed up to 400 mg/kg with SSE maintained growth features and increased health and feed efficiency (Pourhossein et al., 2015). There was no impact on broilers' weight gain or feed intake during the initial growth period (Hajhashemi et al., 2002; Sanbongi et al., 2003; Uslu et al., 2003; Osakabe et al., 2004; Nybe, 2007; Can Baser, 2008; Brown, 2009; Burlando et al., 2010; Cutler et al., 2010; Rodov et al., 2010; Tavafi and Ahmadvand, 2011; Akhalkatsi et al., 2012; Gelovani et al., 2012; Hashemipour et al., 2014) (Ghazvinian et al., 2018). During the 15–28-day growth phase, broilers were fed up to 300 mg/kg SSE, and the feed intake was gradually lowered (Ghazvinian et al., 2018). During the finisher phase (Adiguzel et al., 2007; Brighente et al., 2007; Kizil et al., 2009; Mahboubi and Kazempour, 2011; Hamidpour et al., 2014; Aadil et al., 2015; Cvetanović et al., 2017; Katar et al., 2017; Mašković et al., 2017; Mohtashami et al., 2018; Maqbool et al., 2022; Arshad et al., 2023; Fatima et al., 2023; Waliat et al., 2023), dietary interventions had no effect on any of the growth indices examined. Finally, dietary supplementation with summer savory extract at 400 mg/kg as a natural feed addition helped broiler chickens maintain their growth and enhance their health (Daferera et al., 2000). (Figure 5).

6 Summer savory and toxicity

Toxicity is a condition in which chemical compounds or parts of a chemical mixture can damage an organ. Antioxidants are consumed on a large scale as nutraceuticals and food supplements that can preserve optimal health. It is well known that "excess of everything is bad", yet it is not generally recognized that a high intake of antioxidants may also have adverse effects. On the other hand, some antioxidants are used to illustrate general considerations on the toxicity of antioxidants. The

toxicity of antioxidants can be evaluated with some recommendations, including classical safety factors, the knowledge of the efficacity mechanism, bio-kinetic/bio-efficacy modeling, and antioxidant supplementation changes into therapy being also of interest (Bast and Haenen, 2002). The study of Boroja et al. (2018) suggests that the methanolic extract of the summer savory aerial part protects against cisplatin-induced oxidative damage. Cisplatin was induced to produce toxicity using intraperitoneal injection (Boroja et al., 2018). The results confirm that summer savory could be a valuable source of dietary and therapeutic phenolic compounds. However, summer savory can maintain normal health conditions or may be a remedy for different oxidative damage diseases (Boroja et al., 2018). Furthermore, there are fewer known side effects of *S. hortensis*, but people who are taking medication for chronic diseases are cautioned about its use. The advice of a healthcare provider is mandatory before starting any new therapy or consumption of medicinal plants. *S. hortensis* is not recommended for children, pregnant women, or breastfeeding women due to a lack of sufficient evidence of its safe use in these populations (Hamidpour et al., 2014). In general, essential oils are comprised of a large variety of elements; hence, they do not appear to have any unique cellular targets. The presence of phenols, aldehydes, and alcohols in essential oils contributes to their cytotoxic effect (Bakkali et al., 2008). It appears that a distinction can be made between *Satureja* spp. and poisonous effects in eukaryotic cells and cytotoxic effects on microorganisms (yeast, viruses, fungi, and bacteria). The anti-pathogenic action of *Satureja* spp. has been well documented. Many people throughout the world consume *Satureja* spp. in the form of spice, herbal tea, and extracts. *S. hortensis* is consumed as a vegetable on a daily basis and has no known negative effects (Hajhashemi et al., 2000). Furthermore, an *in vitro* study showed that both the ethanolic extract and the essential oil of summer savory protected rat lymphocytes from hydrogen peroxide-induced damage (Behravan et al., 2006). In rats, the essential oil of *S. khuzestanica* was found to protect against the toxicity of Malathion (a commonly used organophosphorus) (Basiri et al., 2007). The leaves of *S. gilliesii* contain two isomeric monoterpene peroxides and they were found to be poisonous to *Artemia salina* in other investigations (brine shrimp bioassay) (Labbe et al., 1993).

7 Conclusion

It is concluded that the leaves and seeds of summer savory (*Satureja hortensis* L.) contain different chemical components. Summer savory leaves are abundant in total phenolic components, especially flavonoids and rosmarinic acid. It has strong antioxidant, antimicrobial, and antifungal effects that play a preventative role in human health. Furthermore, their oxidant activity suppresses the growth of many large tumor cells and the growth of HT-29 (human colon adenocarcinoma) cells. Carvacrol and Thymol are suppressing a rich diversity of microbes in *S. hortensis*, which have medicinal properties such as anti-diabetic, antispasmodic, anti-hyperlipidemic, anti-inflammatory, anti-proliferative, and anti-nociceptive properties.

Author contributions

Conceptualization, MA and AE; methodology, WK; software, WK; validation, SW, WK, and MK; formal analysis, HR; investigation, HR; resources, M-IL; data curation, WK; writing—original draft preparation, AE; writing—review and editing, SW, MU-I, and IC; visualization, M-IL, AB, and CM; supervision, MA., SM; project administration, SM; funding acquisition, SM. 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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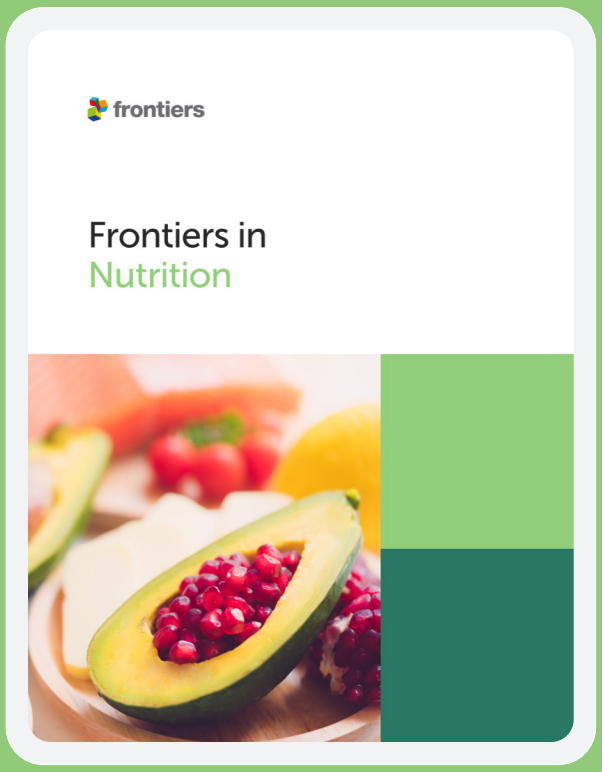

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