Research Topics

Technological advancements for processing and preservation of fruits and vegetables

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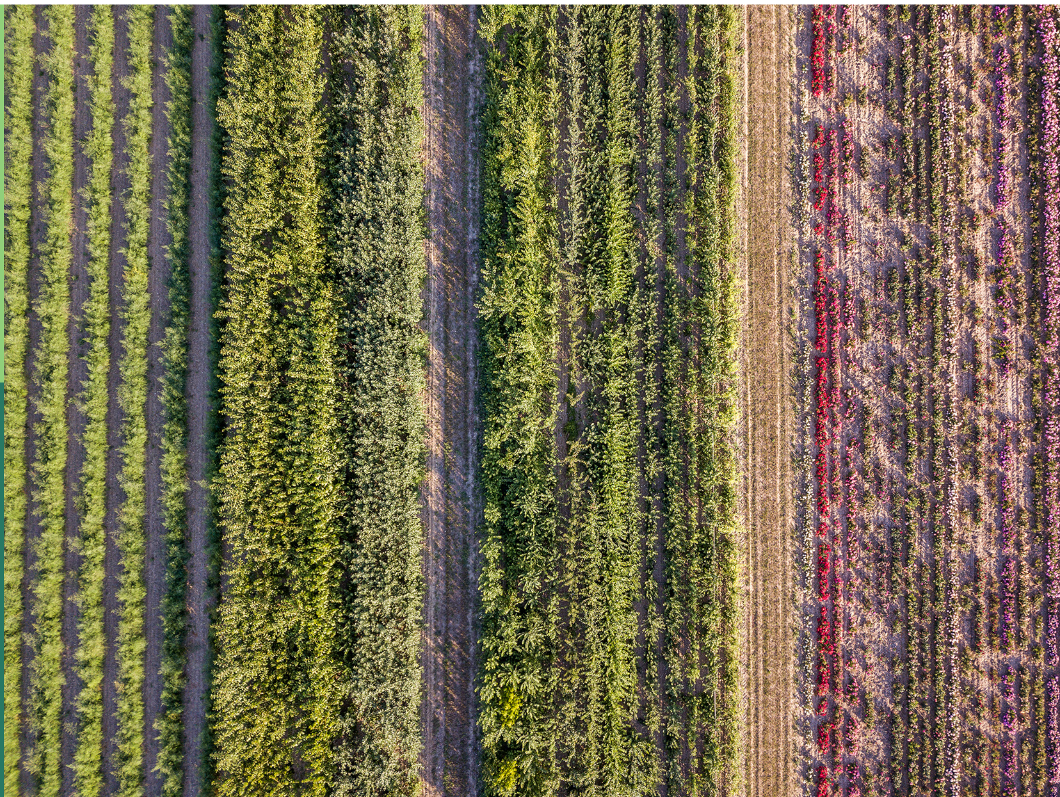

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Technological advancements for processing and preservation of fruits and vegetables

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Table of contents

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Editorial: Technological advancements for the processing and preservation of fruits and vegetables

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Editorial on the Research Topic
Technological advancements for the processing and preservation of fruits and vegetables

Fruits and vegetable-rich diets contain plenty of vitamins, dietary fiber, and minerals, particularly bioactive compounds and antioxidant activities, which are advised for their health-boosting effects. Toxicological, epidemiological, and nutritional studies proposed the relationship between fruit and vegetable intake and a lower prevalence of diseases, including cancer, heart problems, Alzheimer's, and diabetes. New products, processing methods, and distribution conditions will increase fruit and vegetable consumption. Recently, transformations have occurred as many food processing and preservation technologies evolved and advanced, particularly within the agro-food sector. Undoubtedly, all have mainly transpired due to evolving consumer needs for fresh and highly nutritious foodstuffs with improved shelf life. Since consumers are influential in the agro-food sector, consumer needs have been a vital motivation to other parties in the agro-food industry, including food scientists, who are paramount in ensuring the quantity and quality of foods. The current research issue strives to contribute to plugging the research gap in the knowledge regarding technological advancements for fruit and vegetable processing and preservation. In this context, fourteen papers were published; nine are original research papers, and the rest of the five are reviews.

In review, Hassan et al. unveil the potential of physically stimulating methods for seed invigoration to increase the sprouting rate, such as laser irradiation, gamma irradiation, microwaves, plasma, magnetic fields, ultrasonic waves, and sound waves. Physical methods offered different advantages than conventional methods, including high germination rate, environment-friendly, early seedling emergence, protection from chemical hazards, uniform seedling vigor, and enhanced yield. In the second review, Bhatti et al. proposed an in-depth knowledge of the antibacterial properties of *Nigella sativa, capsicum, Musa paradisiaca* L. peels, and *Citrus limetta* with its potential food applications. Also, the review summarizes the advantages and disadvantages of each novel extraction technique applied to extract these antimicrobial agents. Shaukat et al. unveiled the recent progress on tea polyphenols, their potential health benefits, and effective traditional and modern techniques to extract polyphenols. Ragu and Teo stated the art of available few-shot learning models, networks, and classifications and proposed insights into future research routes. Few-shot learning shows higher accuracy in vegetable disease detection in a limited dataset. They also revealed that few-shot learning is trustworthy with few instances and less training time. Yépez-Ponce et al. stated the potential use of mobile robotics in farming to address environmental impact, productivity, crop losses, food safety, and sustainability challenges. The existing agricultural robotic systems are expensive and large because they use a computer as a server and mobile robots as clients. One technological solution that could be executed is designing a fully independent, low-cost agricultural mobile robotic system without a server.

About the original research, He et al. developed enzymolysis and microwave-assisted enzymolysis methods to improve the water-holding capacity of kombucha cellulose hydrolysates while lowering the oil-holding capacity. *In vitro,* digestion outcomes proposed substituting kombucha cellulose hydrolysates would have no harmful health impacts in the deep-fried donut formula. Saleem et al. studied the effect of freeze-drying on mung bean peel powder and soybean peel powder and their incorporation into functional yogurt at different concentrations. The results revealed that the physicochemical profile of functional yogurt had a linear proportional relationship with both powder concentrations. When powder concentration increases, bioactive compound, antioxidant activity, and rheological properties significantly improve with better sensory acceptability. Results offer that soybean and mung bean peel could be utilized in food products as a source of bioactive compounds after freeze-drying. Wiset et al. established a combined method of microwave drying with hot air drying at 45°C, successfully preserving the bioactive compounds, volatile organic compounds, and overall quality of the two cultivars of holy basil leaf for pharmaceutical and food industries. This alternative drying method is viable for drying red and green holy basils with better product qualities, better bioactive compound retention, and lower electrical energy consumption at industrial scales.

Khalil et al. evaluated the *Terminalia chebula* varieties (black, Kabuli, and green), revealing that the Kabuli and green varieties possess better minerals and proximate composition than the black variety. In muffin development, the 7.5% level of all three showed better acceptability. The current study has proved that white flour supplementation with *Terminalia chebula* varieties develops functional muffins. Suthiluk et al. reported the potential of pomace from "Phulae" pineapples for making new functional snacks due to their physicochemical properties. Employing the Box–Behnken design allowed the determination of optimum parameters for processing pineapple pomace-fortified crispy mushroom sheets with maximum antioxidant activities and soluble dietary fibers. The results of the present study revealed that crispy sheet mushroom products are functional food and a better alternative protein snack fortified with dietary fiber. Mthembu et al. investigated the efficacy of hexanal-based enhanced freshness formulation, having antioxidants such as ascorbic acid, α-tocopherol, and geraniol on preserving the bioactive components of gold kiwifruit harvested at two maturity stages. The results revealed that enhanced freshness formulation significantly affected antioxidant capacities, bioactive compounds, and the enzyme activity entangled in the synthesis and oxidation of bioactive compounds. The maturity stage significantly impacts the bioactive compounds.

Yang et al. proposed the Kalman filter fusion algorithm, an online monitoring system for real-time monitoring of the moisture content in an air-impingement dryer. It was employed to evaluate the optimal state of the original detection values of the weighting sensor and air velocity sensor. This monitoring system has better detection accuracy for moisture content in the drying process and also improves the automation level of the drying equipment for fruits and vegetables. The suggested Kalman filter fusion algorithm also provides a reference for other multifactor fusion detection. Karacan et al. unveiled that food costs and food shortages caused by population growth and climate change, along with the drought, make it more critical to prevent food losses with the across-the-board use of cold storage. In the current study, the authors examined the energy consumption data of cold stores obtained from different countries. Obtained results revealed that cold storage energy consumption increased by up to 30%, based on operational conditions. Thus, cold stores must be examined by the concerned department during their operation. Gondal et al. proposed a blockchain-based food supply chain solution for the food supply chain industry. The proposed methodology is the combination of blockchain technology and the Internet of Things, brilliant contracts, to ensure the highest quality delivery of strawberries. The Internet of Things-enabled container provides information about the quality of the strawberry, humidity, temperature, and shipment location.

Conclusively, the scientific efforts led to the utilization of new food processing technologies without high heat processing involvement, including microwaves, spray drying, and ohmic heating, to better retain nutritional quality. Also, novel non-thermal processing technologies, such as ultrasound, irradiation, pulsed electric field, high-pressure processing, cold plasma, and freeze-drying, proved promising for processing fruits and vegetables. Likewise, spray drying is employed for slurry or liquids using hot gas. This method is preferable for thermally sensitive materials, while freeze-drying is used for rehydrated products without changing the product's original shape.

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MM: Conceptualization, Data curation, Funding acquisition, Supervision, Writing—original draft, Writing—review & editing. RS: Data curation, Writing—original draft, Writing—review & editing. AH: Writing—original draft.

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Recent developments in physical invigoration techniques to develop sprouts of edible seeds as functional foods

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For nutritional security, the availability of nutrients from food sources is a crucial factor. Global consumption of edible seeds including cereals, pulses, and legumes makes it a valuable source of nutrients particularly vitamins, minerals, and fiber. The presence of anti-nutritional factors forms complexes with nutrients, this complexity of the nutritional profile and the presence of anti-nutritional factors in edible seeds lead to reduced bioavailability of nutrients. By overcoming these issues, the germination process may help improve the nutrient profile and make them more bioavailable. Physical, physiological, and biological methods of seed invigoration can be used to reduce germination restraints, promote germination, enhance early crop development, to increase yields and nutrient levels through sprouting. During sprouting early start of metabolic activities through hydrolytic enzymes and resource mobilization causes a reduction in emergence time which leads to a better nutritional profile. The use of physical stimulating methods to increase the sprouting rate gives several advantages compared to conventional chemical-based methods. The advantages of physical seed treatments include environment-friendly, high germination rate, early seedling emergence, uniform seedling vigor, protection from chemical hazards, and improved yield. Different physical methods are available for seed invigoration viz. gamma irradiation, laser irradiation, microwaves, magnetic field, plasma, sound waves, and ultrasonic waves. Still, further research is needed to apply each technique to different seeds to identify the best physical method and factors for seed species along with different environmental parameters. The present review will describe the use and effects of physical processing techniques for seed invigoration.

KEYWORDS

seed germination, gamma irradiation, laser irradiation, microwaves, magnetic field, plasma, sound waves, ultrasonic waves

Introduction

Edible seeds including cereals, pulses, and legumes are globally used as a staple food and possess several functional properties like antioxidant, antidiabetic, anticancer, and antitumor effects. They are a rich source of vitamins, minerals, and fiber and also contain enough amount of bioactive components such as phenolics, carotenoids, lignin, β-glucan, inulin, resistant starch, sterols, and phytates. According to several studies, the controlled germination process is a valued technique to improve the nutritional and medicinal values of edible seeds (Hayat et al., 2014; Verspreet et al., 2015; Özer and Yazici, 2019) (Figure 1). Sprouts are germinated seeds of cereals, pulses, and legumes that grow into seedlings, and are characterized by nutrient bioavailability and their profile including phenolic profile, antioxidant profile, vitamins, minerals, along with other micro and macronutrients. As compared to the un-sprouted grains the sprouted grains are considered an important functional ingredient due to having major nutritional, textural, and tasteful, advantages. The edible sprouts can help to provide essential nutrients, maintain health status, and to prevent disease. Currently, interest in the use of sprouted grains as functional ingredients and food is growing with increased interest from food researchers, nutritionists, producers, and consumers (Aloo et al., 2021; Pires et al., 2021).

The germination process can improve the levels of simple sugars, free amino acids, and organic acids through the catabolism of macronutrients like carbohydrates, protein, and fatty acids (Wang et al., 2005; Shi et al., 2010; Benincasa et al., 2019). It can also decrease different anti-nutritional factors and indigestible components, like lectin and protease inhibitors (Saithalavi et al., 2021). Moreover, through germination secondary metabolites such as vitamin C and polyphenols can accumulate in edible seeds (Toro et al., 2021).

Sprouting is the crucial phase in the plant at which fast germination and seedling emergence are vital aspects that can be stimulated in seeds (Sharififar et al., 2015; Lai et al., 2016). Germination stimulation has been achieved through several methods such as fertilizers, light, seed scarification, seed stratification, salinity, temperature, humidity, and regulatory hormones (Rifna et al., 2019b). As a substitute for chemical invigoration techniques for plant growth stimulation, the use of physical methods attracts more and more attention. The physical techniques can improve food quality without imparting any safety concerns, thus the applications of these methods have increased to affect plant growth and germination (Bose et al., 2018).

Now sprouted grains are becoming popular and consumed all over the world. To increase the nutritional value of foods and to improve the sensory properties of sprouted grains flour is used in different products. Despite several efforts to explore the utilization of sprouted grains, limited products have been produced and introduced into the market. Thus, it is required to conduct more research and incorporate seed sprouts into different food industries to introduce them into the food chain (Liu et al., 2017; Yilmaz et al., 2020).

Therefore, different physical methods have been developed to facilitate the germination process and to improve the plants' growth and production. This paper gives a brief overview of such physical processing technologies including gamma irradiation, laser irradiation, microwaves, magnetic field, plasma, sound waves, and ultrasonic waves. Additionally, mechanisms of seed germination promotion by processing treatments and how they impact germination have also been discussed.

Physical seed invigoration techniques

Throughout the transient activation of the pre-germination metabolic activities, seed priming is a well-known and established way to improve seed quality (good nutritional composition along with reduction of seed dormancy, the breakdown of the complex of anti-nutrients, and the release of nutrients and their improved bioavailability) (Chakraborti et al., 2021). Under adverse environmental circumstances (abiotic stresses like water deficit, high salinity, high temperature, submergence, etc.) seed priming has arisen as a constructive way of improving seed vigor, germination synchronization,

Abbreviations: MWs, Microwave radiations; US, Ultrasonic waves; IR, Infrared; WLAN, Wireless router; MFs, Magnetic fields; DNA, Deoxyribonucleic acid; ROS, Reactive oxygen species; RF, Radio-frequency; SMF, Static magnetic field; EMF, Electric magnetic field.

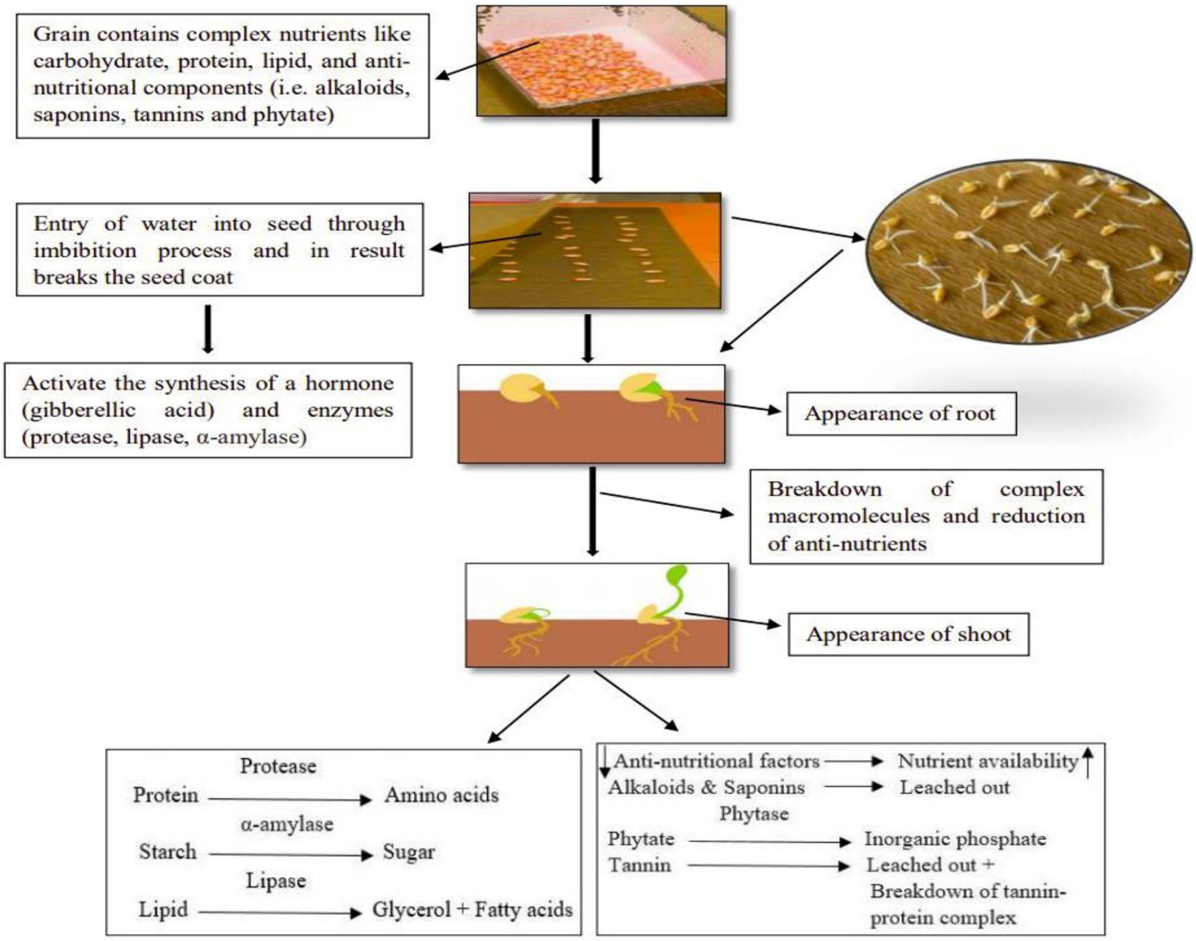

FIGURE 1
Schematic diagram of the germination process.

and seedling growth (Marthandan et al., 2020). A variety of priming techniques is available, and some of them are properly categorized such as hydro-priming and osmo-priming. As compared to osmo-priming and chemical-based conventional treatments, physical processing techniques have shown several advantages. Due to their less damaging effects on the environment like anthropogenic changes in the soil, water, and atmosphere, recently the use of physical methods for plant growth stimulation is becoming more popular (Table 1) (Bilalis et al., 2012; Rifna et al., 2019a). In chemical-based methods, the required chemical compounds are directly injected into the cell while in physical methods, energy is introduced into the cell which generates conditions for different transformations at a molecular level (Govindaraj et al., 2017). Different positive biological changes can be introduced in plants without influencing their biology through the application of various physical factors. These physical techniques decrease the on-farm pollution of raw materials, minimize the requirement for fertilizers, and can also be used for the disinfection of seeds (Table 2) (Bera et al., 2021). All biological activities depend on the exchange of energy between the cell and the environment. Improving the germination and yield of crops by using energy is an advanced area in research. Energy treatment stimulates the enzymatic reactions leading to the initiation of physiological and biochemical changes. All these changes are an indication of plant growth and development processes which eventually improve the quality and yield of produce (Govindaraj et al., 2017).

Ultrasound seed processing

Ultrasonic waves (US) are mechanical waves having a frequency higher than 20 kHz and cannot be detected by a human audition system. This technology has been successfully used in different mass transfer processes of food including drying, extraction, osmotic dehydration, desalting,

TABLE 1 Different physical techniques used for the seed invigoration.

Technique	Principle	Mechanisms	References
Ultrasound	Lead to cavitation phenomenon exerting mechanical pressure on the seeds	• The fluidity of the cell wall • Formation of cracks and micropores on the cell wall • Enhanced exchange of water and oxygen	Hu et al., 2007 Miano et al., 2016
Microwave heating	After absorption induces ionic movement and dipole rotation	• Deformation of the electron orbits • The fast and selective heating process • Electronic transitions between different rotational sublevels	Mullin, 1995 Al Mashhdani and Muhammed, 2016
Magnetic field	The perception and signaling mechanism is mediated through the blue light photoreceptors called cryptochromes	• Induce variations in the ionic concentrations and membrane potential • Increased water uptake	Shine et al., 2011 Socorro and García, 2012
Plasma treatment	Reactive oxygen species (ROS) in water vapor plasma influenced the redox reaction	• Affect the plant development by controlling thiol groups • Thin layers of hydrophobic and hydrophilic nature are produced	Volin et al., 2000 Henselová et al., 2012
Gamma irradiations	Reactive oxygen species (ROS) as the main regulators produced in the seed	• Activate and amplify stress and antioxidant responses • Affect nucleic acids and proteins synthesis leading to metabolic activities	Borzouei et al., 2010 Esnault et al., 2010
Sound waves	Enhanced the transcription level and activate the stress-induced genes	• Stimulate the opening of leaf stomata • Sound waves converted into or reserved as chemical energy • Stimulate the photosynthetic reactions	Meng et al., 2012 Xiujuan et al., 2003
Laser irradiation	Synergistic effect between the polarized monochromatic laser beam and the photoreceptors	• Coherent laser light caused illumination of biological tissues and speckle formation • Strong intensity gradients in the tissues • Induce inter-and intracellular gradient forces • The paths and speeds of biological processes significantly changed	Ruvinov, 2003 Hernandez et al., 2010

and hydration (Miano et al., 2016; Asfaram et al., 2019). Nowadays to break the seed dormancy ultrasonic waves have attracted the researcher's attention as being a safe, easy, and time-saving technique (Ramteke et al., 2015; Liu et al., 2016). In recent years the mechanism of ultrasonic wave activity on seed germination in different plant species has been explored as mentioned in Table 3. In ultrasonic treatment, seeds are placed into an ultrasonic wave emitting apparatus in which water is used as a medium (Nazari and Eteghadipour, 2017). For seed germination, oxygen availability and water uptake are the essential parameters, so ultrasonic waves alter the seed's characteristics through which these factors become available more efficiently (Liu et al., 2016).

Ultrasonic waves in water lead to cavitation, a phenomenon creating micro-bubbles in water that exerts mechanical pressure on the seeds. Mechanical pressure exerted by the cavitation process further causes fluidity of the cell wall and the formation of cracks and micropores in it (da Silva and Dobránszki, 2014; Rifna et al., 2019a). A study conducted on mung beans showed an increase in their porosity after ultrasonic treatment. Seeds become more porous for water and oxygen exchange due to the production of micro-pores and micro-cracks. It was demonstrated that ultrasound technology improved the hydration process of mung beans, reducing the total process time by almost 25% [increasing the water absorption rate to ∼44%] (Miano et al., 2016). In studies, conducted by Yaldagard et al. (2008) and Sharififar et al. (2015) it has been revealed that ultrasonic treatment increases the hydration process in seeds, therefore leading to an increase in enzymatic reactions especially related to alpha-amylase. Thus, the starch hydrolysis conducted by alpha-amylase has resulted from an increment in seed germination speed and percentage (Yaldagard et al., 2008; Sharififar et al., 2015). A schematic presentation of ultrasonic wave treatment in seeds is shown in Figure 2.

Through several studies, it has been investigated that ultrasound is a promising method to break seed dormancy and enhance germination. A summary of the findings regarding the effects of ultrasound treatment on the seed germination percentage of different edible seeds has been provided in Table 3. Yaldagard et al. (2008) reported a 6% increase in germination

TABLE 2 Advantages and limitations of novel processing techniques.

Technique	Advantages	Limitations	References
Ultrasound	• Safe, easy, and time saving • Induce mechanical pressure on seeds • No chemical contamination	• Has a small size of apparatuses • Scaling-up is required to manufacture huge ultrasound demitting sets	Nazari et al., 2014 Ramteke et al., 2015 Liu et al., 2016 Nazari and Eteghadipour, 2017
Microwave heating	• The short startup, precise control, and volumetric heating • Have the fast and selective heating ability	• Under field conditions, the uneven temperature distribution is one of the problems	Warchalewski et al., 2011 Brodie, 2012 Motallebi, 2016
Magnetic fields	• Less toxicity • Easy to manipulate • Cost-effective and safe method • Helpful to overcome the effect of salt stress	• Having an impact on seed recovery applications when seeds have low quality, for specific plant species	Balouchi and Sanavy, 2009 Rácuciu, 2011
Plasma treatment	• Low temperature and treatment duration • Can be used for thermally sensitive materials • Appropriate for a large range of materials and shapes • Absence of potentially environmentally-harmful chemicals • Avoidance of toxic reagents or by-products	• Low-pressure radio frequency plasma systems have limitations in terms of environmental and economic costs • Also has processing restrictions regarding vacuum processing	Filatova et al., 2009 Šerá et al., 2009 Ling et al., 2014 Zhou et al., 2016
Gamma irradiations	• Cause small variations in food components • Requires minimal sample preparation • No use of catalyst • Excellent penetration • Causes no increase in temperature during processing	• Requires optimization of the treatment parameters including temperature, exposure time, and dose	Selcuk et al., 2008 Grover and Khan, 2014 Bashir and Aggarwal, 2016
Sound waves	• Reduce resource usage • Decrease the requirements for chemical fertilizer and pesticide	• Causes noise pollution and confusion • Have contradictions in terms of frequencies and exposure periods • The sound pressure level falls inversely proportional to the distance from the sound source	Carlson, 2013 Hassanien et al., 2014
Laser irradiation	• Suitable to radiate a large number of seeds • Diodes have low costs • Avoid the use of harmful fungicides	• Laser beams are narrow • The whole surface of the seed is not evenly exposed	Claudia et al., 2011 Sharma et al., 2015

percentage of barley (*Hordeum vulgar L.*) seeds after ultrasound waves' treatment as compared to control. Application of sonication treatment on Norway spruce (*Picea abies L.*) Karsten seeds increased germination by 22% (Rîşca and Fártáiş, 2009). Goussous et al. (2010) showed that ultrasonic waves' application to chickpeas (*Cicer arietinum*), wheat (*Triticum aestivum*), and watermelon (*Citrullus vulgaris*) increased their germination percentage by 36, 2, and 2%, respectively, in comparison to control. Another investigation conducted by Aladjadjiyan (2011) revealed a 4 and 6% increase in the germination of wheat (*Triticum aestivum*) and lentils (*Lens culinaris, Med.*), respectively. According to an investigation by Wang et al. (2012) on switchgrass (*Panicum virgatum L.*) seeds, sonication enhanced germination by up to 23.2%.

The application of ultrasonic waves on sunflower (*Helianthus annuus L.*) seeds, enhanced their germination maximum by up to 43.38% (Machikowa et al., 2013). Another *in vitro* study conducted on snail medick [*Medicagoscutellata* (L.) *Mill*] seed indicated that ultrasound increased germination up to 63.3% (Nazari et al., 2014). In a study conducted on peas (*Pisum sativum*), as compared to control, sonication treatment caused a 13.1% increase in seed germination. The operating parameters were time and temperature at specific input power. Pea seeds were subjected to an ultrasonication treatment of 40 kHz for 1 min at 25 celsius (Chiu and Sung, 2014). A similar study conducted by Sharififar et al. (2015) showed that ultrasound treatment applied to big saltbush (*Atriplex lentiformis*), cumin (*Cuminum cyminum*), and caper

TABLE 3 Effect of ultrasonic waves on seed germination (%).

Plants	Working conditions	Control germination	Ultrasound germination	References
Barley (*Hordeum vulgare*)	Frequency 20 kHz, Wave amplitude 210 µm, Power 460 W	93%	99%	Yaldagard et al., 2008
Chickpea (*Cicer arietinum*)	Frequency 40 kHz, Output 100 W,	61%	97% 100%	Goussous et al., 2010
Wheat (*Triticum aestivum*)	Power supply 220 V 50 Hz	98%		
Wheat (*Triticum aestivum*)	Frequency 42 kHz, Power 100 W	90%	94% 98%	Aladjadjiyan, 2011
Lentil (*Lens culinaris*)		92%		
Sunflower (*Helianthus annuus*)	Frequency 40 kHz, Power 250 W	54.6%	98% 68%	Machikowa et al., 2013
Norway spruce [*Picea abies (L.) Karsten*]		46%		
Snail clover [*Medicago scutellata (L.) Mill*]	Frequency 42 kHz	33.3%	96.6%	Nazari et al., 2014
Saltbush (*Atriplex lentiformis*)	Frequency 42 kHz	40%	68% 80%	Sharififar et al., 2015
Cumin (*Cuminum cyminum*)		44%		
Russian wildrye (*Psathyrostachys juncea Nevski*)	Frequency 40 kHz, Power 200–500 W	39.3%	89.3%	Liu et al., 2016

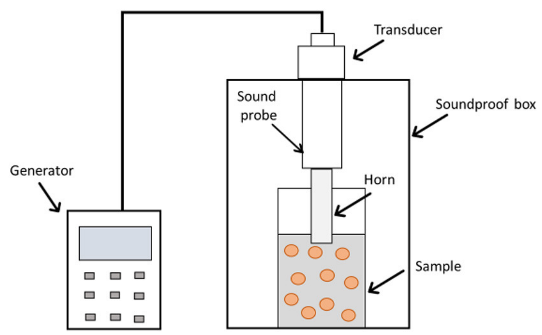

FIGURE 2
Schematic diagram of the ultrasound seeds processing.

beans (*Zygophyllum eurypterid*) significantly increased their germination percentage up to 28, 36, and 35.7%, respectively, in comparison to control treatment. Overall in agreement with the positive effects of ultrasonic waves studied in most cases of tested species, it's clear that ultrasonic waves can affect seed germination positively.

Microwave seed treatment

The microwave component of the electromagnetic spectrum includes radiation having a frequency within the range of 300 MHz to 300 GHz and wavelength ranging between 1 m down to 1 mm. Now it is recognized that microwave radiation (MWs) after absorption as non-ionizing electromagnetic radiation causes different changes in biological systems which are mostly thermal and non-invasive (Bera et al., 2021). MWs can induce various biological changes depending on different factors such as field strength, frequencies, waveforms, modulation, and duration of exposures (Vian et al., 2006). Mostly the effect of MWs on humans and animals was extensively studied and addressed, while there is a very small number of studies related to the effect of MWs on plants (Jayasanka and Asaeda, 2013). Most of the available work described the effect of radiations having the 2.45 GHz frequency, which is absorbed in living cells through water molecules (Creţescu et al., 2013). After absorption into living cells and tissues, MW radiations induce ionic movement, and dipole rotation leading to the deformation of the electron orbits which finally causes a fast and selective heating process (Mullin, 1995; Rifna et al., 2019a). The schematic diagram of the experimental set-up for microwave treatment is given in Figure 3.

MWs treatment can also result in electronic transitions between different rotational sublevels. In the organic molecules between vibrational levels transitions mostly occur in near Infrared (IR) regions (750 to 1,300 nm) of the electromagnetic spectrum, while between rotational levels occur in far IR regions and near microwave regions (1 mm to 1 m) (Al Mashhdani and Muhammed, 2016). Intermediated frequency levels of radiation (2,450 MHz) applied to seeds showed higher enzymatic reactions and increased growth rates. Still, the mechanism of MWs is not as yet fully understood but according to Rajagopal (2009) exposure to microwaves, 2.45 GHz and 650 W for 30 s are enough to ensure a high germination rate. In several studies, lethal level MWs have been used for preventing the growth of weeds in the soil while non-lethal level MWs treatments have been widely used for seed decontamination before sowing (Scialabba and Tamburello, 2002; Knox et al., 2013; Sahin, 2014). Application of MWs caused heating of soil up to 80°C which results in suppressed germination of the weed. Thus, in greenhouses from horticultural/ornamental plant nurseries,

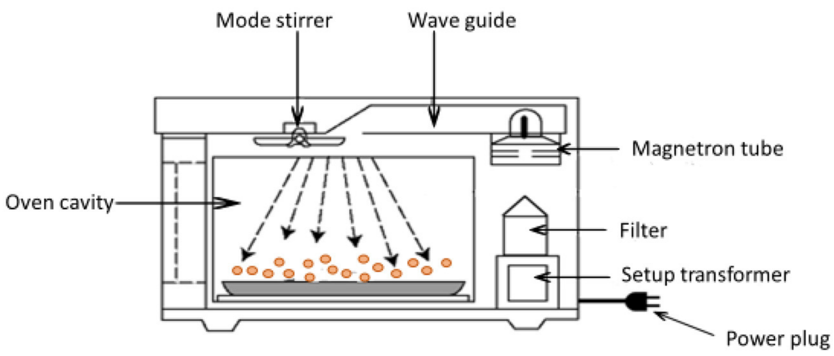

FIGURE 3
Experimental set-up for microwave exposure.

TABLE 4 Effect of microwaves waves on germination parameters.

Plants	Working conditions	Effects	References
Wheat (*Triticum aestivum*)	Frequency 2.45 GHz, Power 750 W	• Reduced seed vigor • Seed-borne infestation of Fusarium graminearum decreased	Reddy et al., 1998
Radish (*Raphanus sativus*)	Frequency 10.5 and 12.5 GHz, Power 8 and 14 mW	• The reduction in germination % and rate • Reduced hypocotyl growth	Scialabba and Tamburello, 2002
Lentil (*Lens culinaris*)	Frequency 2.45 GHz, Output power 450 and 730 W	• Seed germination % and rate not affected • Seedling length stimulation	Aladjadjiyan, 2010
Potato (*Solanum tuberosum*)	Frequency 38, 46 and 56 GHz, Output power 4 mW	• Increased biomass growth	Jakubowski, 2010
Barley (*Hordeum vulgare*)	Frequency 2.45 GHz, Output power 800 W	• Increased germination and vigor index	Creţescu et al., 2013
Rice (*Oriza sativa*)	2,450 MHz	• Germination % and rate enhanced • Increased length of primary shoot and root	Talei et al., 2013

MWs appeared as an effective non-chemical alternative method for weed management (Velázquez-Martí et al., 2006).

There is very limited literature about the use of MWs radiations as seed stimulation treatment in a few plants, affecting their germination performance. Through different studies, it was investigated that application of 2.45 GHz MWs radiation has no major influence on seed germination, but in some plant species including wheat, green gram, moth bean, and Bengal gram, it showed a beneficial effect on biomass accumulation and growth (Jakubowski, 2010; Talei et al., 2013). A summary of the findings of the effects of microwave treatment on seed germination of the mentioned plant species has been provided in Table 4. The effect of microwave irradiation at 935.2–960.2 MHz with intensities of 0.07–0.15 mW/cm^2 on maize grains was studied and revealed a clear increase in germination and seedlings development (Khalafallah and Sallam, 2009). Aladjadjiyan (2010) conducted an experiment stating that microwave pretreatments with frequency 2.45 GHz for 5, 10, 15, 20, and 25 s and seeds showed enhanced germination parameters as compared with controls. Best results were obtained with an exposure time of 30 s and output power of 450 W giving 10% longer shoot length and 7% root length than the control one. Ragha et al. (2011) used the low power MWs (frequency range of 8.5–10.27 GHz) having non-thermal intensity having a frequency of 1 kHz and studied their effect on the wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum*), green gram (*Vigna radiate*), and moth bean (*Vigna Aconitifolia*). Effects of different parameters like frequency (8.5 to 10.27 GHz), power (−1.0 to 3.5 dBm), exposure time (12 to 28 min), and power density (1.5 to 5.5 cm) were studied to evaluate their effect on germination. As compared to control the different treatments induced stimulating effects on germination %, seedling vigor, and biomass % of plants including wheat, green gram, moth bean, and Bengal gram, especially when used with low levels of power, exposure time, and power density while high frequency stimulated seed germination as compared to control (Ragha et al., 2011).

In an experiment effect of MWs using a wireless router (WLAN: 70 mWm^{-2}) and mobile devices (GSM: 100 mWm^{-2}) was studied on three different aromatic plant seeds including parsley (*Petroselinum crispum* L. cv. Plained Leaved), celery

(*Apium graveolens* L. cv. Pascal Giant), and dill (*Anethum graveolens* L. subsp. hortorum cv. Common) (Soran et al., 2014). Different plant parameters were studied regarding MWs effect including leaf structure, essential oil content, and emission of volatile compounds. The results exhibited that WLAN frequency MWs appeared to be more harmful than GSM-frequency MWs, and the MWs treatments caused both structural and chemical alternations (Soran et al., 2014). Like other radiation treatments, the efficiency of MWs application depends on different parameters such as plant species, growth stage, exposure duration, frequency, and power density (Jayasanka and Asaeda, 2013). Different studies in this area revealed that MWs treatment showed a positive effect on some plants while negatively influencing other plants; which recommended that the influence of MWs is related to radiation frequency, exposure duration, and environmental circumstances (Khalafallah and Sallam, 2009).

Magnetic fields for seed processing

The use of magnetic fields (MFs) also showed positive responses regarding the rate of germination, growth, and crop yield along with the decreased incidence of pathogenic diseases. In a study annual medics and dodder seeds were treated with an electromagnetic field which shows a significant effect on germination rate. Annual medics seeds were treated at 80 µT for 10 min and 30 min, and 128 µT for 10 min while dodder seeds were treated at 88 µT for 12 h in a wet state, and 128 µT for 24 h in dry seeds (Balouchi and Sanavy, 2009; Araújo et al., 2016). The exposure of MFs depends on flux density and duration of exposure which defines its dose and MFs dose influences the germination, seedling growth, and yield (da Silva and Dobránszki, 2016). The schematic representation of the experimental MFs setup is shown in Figure 4. The flux density of the magnetic field changes with the static or alternating magnetic fields, thus increasing the germination percentage and affecting the preliminary growth stages (Hozayn et al., 2019). In a study, magnetically treated water was used as a hydro-priming technique which as result enhanced the germination rate and plant growth (Morejon et al., 2007). Along with plant growth, the application of MFs also influenced the enzymatic activities, phytochemical reactions, and respiration process (Carbonell et al., 2000; Martinez et al., 2000; Rifna et al., 2019a). MFs treatment results in speeding up the plants' growth, root development, and protein biosynthesis (Kordas, 2002).

The mechanism behind perceiving MFs and then regulating of signal transduction pathway is still not understood. However, according to Ahmad et al. (2007), the mechanism of MFs signaling is facilitated by blue light photoreceptors which are known as cryptochromes. Chloroplast has paramagnetic characteristics therefore, MF treatment induces metabolic reactions in the seed which stimulate the germination (Aladjadjiyan and Ylieva, 2003). Another work performed by Racuciu et al. (2008) showed that the application of a magnetic field enhanced the enzymatic activities. According to Copeland and McDonald (2012), the efficiency of MFs stimulation is assessed by two factors germination energy and germination capacity. Higher germination energy frequently leads to stronger radicle development and increased biomass percentage.

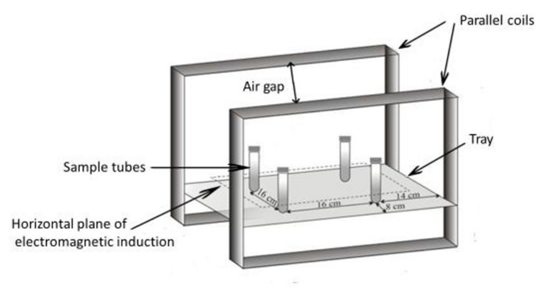

FIGURE 4
The schematic representation of the experimental magnetic fields.

For several years in research studies influence of MFs affecting the plant, and germination parameters have been the subject of interest. Lately, many researchers have stated the positive effects of MFs on germination %, seedling growth, growth of meristem cells, and chlorophyll contents (Qados and Hozayn, 2010; Hozayn et al., 2014). Chickpea (*Cicer arietinum*) seeds were treated with MFs for 1–4 h in steps of 50 mT intensity from 0 to 250 mT and increased germination speed, seedling length, and dry weight as compared to control (Vashisth and Nagarajan, 2008). Static MFs having intensities 4 or 7 mT with 0, 2, 6, and 10 atm osmotic pressure created with sucrose or salt were applied to bean or wheat seeds. The MFs treatment improved the germination ratios, without having any influence of increased osmotic pressure. The greatest germination and growth rates observed in both wheat and bean plants were exposed to 7 mT MF as compared to the untreated seeds. In wheat seedlings, the root and shoot length was 7.63 ± 0.08 and 9.62 ± 0.07%, respectively. In bean seedlings, the root and shoot length was 5.46 ± 0.09 and 7.65 ± 0.08%, respectively (Cakmak et al., 2010). Application of non-uniform MFs having intensities of 60, 120, and 180 mT for different durations of 5, 10, and 15 min, respectively, resulted in significant improvement in pea germination. The high germination leads to increased emergence index, and vigor index by 86 and 205%, respectively (Jamil and Ahmad, 2012). Similarly, in another experiment treatment of corn seed with the pulsed electric magnetic field (EMFs) for different time durations of 0, 15, 30, and 45 min enhanced germination %, vigor, chlorophyll content, leaf area, fresh and dry weight, and yields (Bilalis et al., 2012).

TABLE 5 Effect of magnetic fields waves on seedlings germination parameters.

Plants	Working conditions	Effects	References
Soybean (*Glycine max*)	Magnetic field strength 1,500 nT, Frequency 0.1, 1, 10, and 100 Hz	• Increased protoplasts fusion and germination	Nedukha et al., 2007 Radhakrishnan and Kumari, 2013
Wheat (*Triticum aestivum*)	Magnetic field strength 20 nT-0.1 Mt	• Activation of esterases • Growth reduction	Aksenov et al., 2000
Chickpea (*Cicer arietinum*)	Magnetic field strength 0–250 mT, DC power supply 80 V/10 A	• Improved germination • Increased root length, surface area, and volume	Vashisth and Nagarajan, 2008
Soybean (*Glycine max*)	Magnetic field strength 150 and 200 mT, DC power supply 80 V/10 A	• Reduced level of reactive O_2-radical • Increased Rubisco	Baby et al., 2011 Shine et al., 2011, 2012 Radhakrishnan and Kumari, 2012
Barley (*Hordeum vulgare*)	Magnetic field strength 125 mT	• Increase in length and weight	Martinez et al., 2000
Rice (*Oriza sativa*)	Magnetic field strength 125 and 250 mT 250 mT	• Reduced germination	Flórez et al., 2004
Wheat (*Triticum aestivum*)	Magnetic field strength 4 and 7 mT	• Increased germination • Amyloplast displacement • Higher catalase activity • Low peroxidase activity	Cakmak et al., 2010 Hasenstein et al., 2013 Payez et al., 2013
Mung bean (*Vigna radiata*)	Magnetic field strength 600 mT 600 mT	• Promotion of germination • Malondialdehyde reduction • Increased activity of NO and NOS	Chen et al., 2011 Mahajan and Pandey, 2014
Maize (*Zea mays*)	Magnetic field strength 125–250 mT	• Enhanced germination • Increased fresh weight • Amyloplast displacement • Low hydrogen peroxide and enzymatic activity • Reduced antioxidant activity • Increased stomatal conductance and chlorophyll content	Florez et al., 2007 Turker et al., 2007 Javed et al., 2011 Bilalis et al., 2012 Anand et al., 2012 Shine and Guruprasad, 2012 Hasenstein et al., 2013
Mung bean (*Vigna radiata*)	Magnetic field strength 0.5 µT—75 mT, Power 220 volts	• Improved germination, seed vigor, and starch metabolism	Reddy et al., 2012

After overnight soaking wheat grains were treated for consecutively 4 days and 5 h/day with a 30 mT static magnetic field (SMF) and a 10 kHz EMF. Results showed an increased germination speed and seedling growth compared to the control group (Payez et al., 2013). An MF applied to dormant seeds of barley, corn (*Zea mays*), wheat, and beans significantly enhanced the rate of their seedling growth. Exposure of mung bean (*Vigna radiata*) seeds to static MFs having an intensity of 87 to 226 mT for a duration of 100 min, resulted in a direct enhancement in germination % with increasing MFs intensity. At an intensity level of 0.194 T, the maximum germination of ∼80% was observed as compared to the control (Mahajan and Pandey, 2014). Calculated mean values of germination time, germination rate, germination rate coefficient, magnetic constant, transition time, and water uptake, showed the positive effect of static MF in improving germination (Mahajan and Pandey, 2014). A summary of the findings of the effects of magnetic fields on seedling performance of the different plant species has been provided in Table 5.

Plasma seed treatment

In the agriculture sector, applications of plasma treatment are also gaining attention to influence germination and plant growth (Hayashi et al., 2011; Klämpfl et al., 2012). In various studies, scientists showed improved germination and growth pattern through the application of plasmas with various vapors and gases such as aniline, cyclohexane, and helium, respectively (Jiayun et al., 2014). In this regard, different types of plasma techniques have been used such as atmospheric plasma, microwave plasma, and magnetized plasma (Zhou et al., 2011). Figure 5 represents the schematic setup of the plasma seeds treatment. The influence of different gases used in plasma

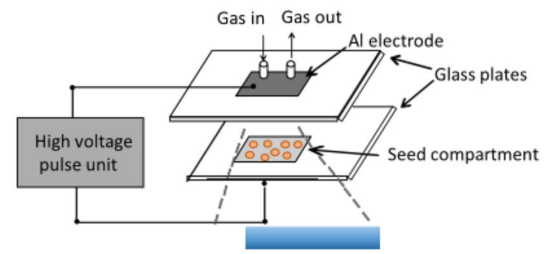

FIGURE 5
Schematic diagram of the plasma seeds treatment.

treatment is generally investigated. Different studies discovered that active oxygen species such as O, O_2^-, O_3, and OH in water vapor plasma influenced the redox reaction which in results affects the plant development by controlling thiol groups' redox status (Henselová et al., 2012). In substitution for scarification and stratification, non-thermal plasma techniques were used as a seed priming method (Dhayal et al., 2006; Mahendran et al., 2017). Plasma application has several benefits including less seed destruction and being environment friendly having no chemical utilization (Volin et al., 2000; Dhayal et al., 2006; Bourke et al., 2018). Besides improving seed quality and plant growth, plasma can sterilize seeds and also cause variations of enzymatic reactions (Sera et al., 2010; Henselová et al., 2012).

Plasma can affect seed germination by suspending or enhancing the process. The new significant plasma-related studies include the use of microwave discharges (Sera et al., 2010) and low-density radio frequency discharges (Bormashenko et al., 2012; Filatova et al., 2013). Plasma treatment induced the development of thin (0.5–2 µm) hydrophobic and hydrophilic layers in the seed, which become very helpful in different cultivation environments including climate conditions, temperature, humidity, lighting, nutrition, and water volume. Thus, in wet and cold soil, the hydrophobic layers interrupt the water absorption which results overcome the chilling injury and improving the seed viability (Volin et al., 2000; Kavak and Eser, 2009). In another study, the use of the plasma method caused increased hydrophilicity which as result stimulated the water uptake and germination process (Bormashenko et al., 2012).

The examination of several seed germination studies showed the effect of different plasma treatments applied on various seeds including wheat, maize, radish, oat, safflower, and blue lupine (Lynikiene et al., 2006; Sera et al., 2010). Different examples from the literature of the plasma treatment on different seeds have been provided in Table 6. Safflower (*Carthamus tinctorium* L. semen) seeds were treated with argon-containing low-pressure capacitively-coupled RF plasma at a pressure of 1.6 and 16 Pa for a time duration of 30 and 130 min, respectively. Treatment of 1.6 Pa, for 30 min resulted in a 30% increase in germination rate while a 50% increase was attained at 16 Pa, for 130 min (Dhayal et al., 2006). The authors claimed that the plasma treatments have caused biochemical modification on the seeds as compared to removing germination inhibitors (Kim, 2019; Guo et al., 2020). Filatova et al. (2013) used air plasma 5.28 MHz at a pressure of 0.3–0.7 Torr to treat blue lupine (*Lupinus angustifolius*), soy, honey clover, and Galega (*Galega virginiana*) seeds and investigated a 10–20% increase in seed germination and crop viability.

Henselová et al. (2012) used a low temperature diffuse coplanar surface barrier discharge air plasma at atmospheric pressure for 60 and 120 s and investigated the growth, anatomy, and biochemical changes that occurred in maize seeds (*Zea mays* L.). After 60 s plasma treatment seedlings showed an increase in root length (21%), root fresh weight (10%), and root dry weight (14%). The authors also detected significant changes in dehydrogenase, superoxide dismutase, catalase, and guaiacol-peroxidase. Bormashenko et al. (2012) treated the grains of lentil (*Lens culinaris*), beans (*Phaseolus vulgaris*), and wheat (*Triticum spp.*) with non-equilibrium plasma. Filatova et al. (2013) and Filatova et al. (2014) treated seeds wheat (*Triticum aestivum* L.), narrow-leaf lupine (*Lupinus angustifolius*), and corn (*Zea Mays* L.) for 10 min with capacitively-coupled low-pressure (40–80 Pa) RF air discharge plasma by using frequency 5.28 MHz and specific power 0.34–0.65 Wcm^3. Similarly, in another study treatment of soybean seeds with cold plasma, treatment having helium with 0, 60, 80, 100, and 120 W for 15 s showed positive effects on seed germination and seedling growth and water uptake was also greater (Ling et al., 2014).

Gamma irradiations seed invigoration

In agriculture sciences, gamma radiation has several applications including food microbiological safety, storability subjects, slow fruit ripening, and vegetable sprouting, along with stimulation of seed germination (Araújo et al., 2016). Among IR, gamma (γ) radiation has high energy and is produced from Cobalt-60. Gamma radiation can penetrate and interact with biological materials (Islam, 2017). Units of Gray (Gy), are used to express the level of absorbed IRs, while 1 Gy dose is equal to 1 Joule radiation energy absorbed per kilogram. Another unit called Sievert unit (Sv) is also used to express the level of absorbed IRs but in the case of interaction biological material, like 1 Gy, 1 Sv is equal to 1 Joule radiation energy absorbed per kilogram of biological material. Another important factor to consider while using the IR technique is the dose rate defined as the rate of energy deposition (Gyh^{-1}) (Moussa, 2006). Gamma radiations can improve product quality, grain yield, and salinity tolerance (Kiong et al., 2008; Majeed et al., 2018). The biological effects of IRs depend on their chemical reactions with biological molecules and water for producing free radicals which control the activity of biomolecules (Araújo et al., 2016). Figure 6 gives

TABLE 6 Effect of plasma treatments on germination parameters of different seeds.

Plants	Working conditions	Effects	References
Corn (*Zea mays*) Soybean (*Glycine max*) Bean (*Phaseolus vulgaris*) Peas (*Pisum sativum*)	• RF rotating plasma reactor • 13.56 MHz • C_4 octadecafluro decalin, aniline, hydrazine, cyclohexane	• Delayed, decreased germination and water uptake for fluorocarbon plasma • Increased germination and water uptake for nitrogen-containing plasma	Denes et al., 2003
Safflower (*Carthamus tinctorius*)	• Radio-frequency (RF) 13.56 MHz • Argon plasma at 20 W	• Increased germination %	Selcuk et al., 2008
Buckwheat [Fagopyrum aescululentum (*L.*) *Moench*]	• Four different plasma treatments for 3, 5, and 10 min. • Glid Arc, planar rotating electrode • At atmospheric pressure, downstream, microwave and dielectric barrier	• Improvement in germination % and lengths of sprouts after Glid Arc treatment	Šerá et al., 2012
Lentils (*Lens culinaris*) Beans (*Phaseolus vulgaris*) Wheat (*Triticum, aestivum*)	• Inductive air plasma discharge • 10 MHz, pressure 6.7×10^{-2} Pa, power 20 W	• Decreased contact angle and germination speed • Increase germination %	Bormashenko et al., 2012
Maize (*Zea mays*)	• Diffuse coplanar surface barrier discharge 10 kV, 14 kHz (sinusoidal) 370 W	• Significantly enhanced root length, root fresh, and dry weight • Root anatomy and morphology are not affected	Henselová et al., 2012
Wheat (*Triticum, aestivum*)	• Atmospheric pressure surface discharge • Room temperature, 15 kV, 50 Hz, 24 W	• Significantly improved root length and dry root weight • A small increment in water imbibition	Dobrin et al., 2015

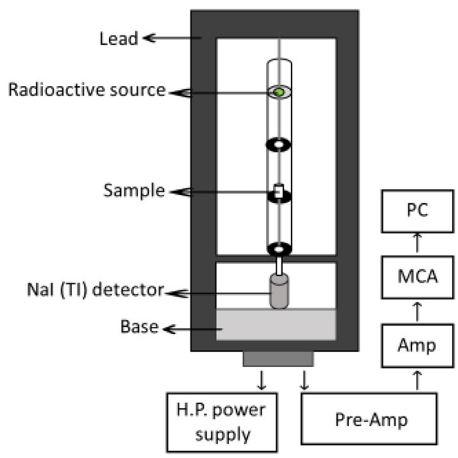

FIGURE 6
Schematic setup of the gamma irradiation treatment.

the schematic outline of the gamma irradiation setup. This leads to the activation of an antioxidant system that prepares the defensive mechanism of plants against stresses (Wi et al., 2007; Ashraf, 2009).

Gamma radiations do not damage the deoxyribonucleic acid (DNA) and structural integrity of seeds and thus can activate various biochemical reactions in the seed (Bhosale and More, 2013). These radiations can affect the different components of seeds like cell membranes, proteins, and nucleic acids. γ-rays can be used as a seed priming technique to boost the germination process but its effect depends on several factors like radiation dose, intensity, and exposure time (Kovacs and Keresztes, 2002; Majeed et al., 2017). The biological or molecular mechanisms involved in the effects of radiation are not properly understood and several ideas have been given.

The application of γ-rays in seeds produces reactive oxygen species (ROS) as the result of water radiolysis. These species react as regulators which can amplify the stress and activate antioxidant responses, thus in this mechanism, ROS has an important role as a signaling molecule [gibberellins signaling pathway and oxidation of negative regulators of germination like abscisic acid] (Borzouei et al., 2010; Esnault et al., 2010). Therefore, γ-rays treated plant seeds easily overwhelmed the fluctuations in daily stress conditions, including light intensity, temperature, and water loss (Gicquel et al., 2012; Qi et al., 2015). Low dose γ-irradiations in seeds induce positive effects on enzymatic reactions and also affect nucleic acids and proteins synthesis which consequently enhances metabolic activities in the seed leading to breaking the seed dormancy and boosting germination speed and plant development (Abdel-Hady et al., 2008). The impact of γ-irradiation in seed technology as a seed invigorating technique has been an impactful way to improve

TABLE 7 Effect of gamma irradiation on germination percentage of different seeds.

Plants	Working conditions	Effects	References
Lentil (*Lens culinaris* Medik)	• Dose rate 1.66 kGy h^{-1} • 0.1–1 kGy	• Reduced germination % up to 40.87% at 0.2 kGy • No germination at 1.0 kGy	Chaudhuri, 2002
Rice (*Oryza sativa*)	• 150–300 Gy	• Decreased germination from 100 to 97.2%	Cheema and Atta, 2003
Maize (*Zea mays*)	• 150, 300, 500, 700, 900, 1,000 Gy • Dose rate 10 Gy /28.97 s	• Germination up to 90% achieved at 240 Gy	Mokobia and Anomohanran, 2005
Long bean (*Vigna sesquipedalis*)	• 300, 400, 500, 600, 800 Gy	• Germination increase up to 70.56% at 400 Gy • No germination at 800 Gy	Kon et al., 2007
Snap bean (*Phaseolus vulgaris*)	• 300, 400, 500, 600, 800 Gy	• Germination decreased from 75.56 to 51.11% • No germination at 800 Gy	Ellyfa et al., 2007
Chickpea (*Cicer arietinum*)	• 100–1,200 Gy • Dose rate 1.66 kGyh $^{-1}$	• Seed germination increased 60–76% with a dose of 100–500 Gy • Germination decreased 80–96% decrease with 700–1,200 Gy	Shah et al., 2008
Wheat (*Triticum aestivum*)	• 100–400 Gy • Dose rate of 0.864 kGy/h	• Germination % decreased from 8.8 to 5.5%	Borzouei et al., 2010
Corn (*Zea mays*)	• 10, 30, 50 kR	• 30% germination at 10 kR dose while untreated showed 50% germination	Itol, 2010

germination. *Arabidopsis thaliana* seeds were treated with γ-ray's 50 Gy dose which in result showed positive results on all the tested growth parameters such as germination index, seedling growth, root length, and fresh weight (Qi et al., 2015).

Similarly, in another study, *Oryza sativa* L. cv-2233 and *Phaseolus mungo* L. dried seeds were treated with γ-rays ranging between 50 and 350 Gy. *Oryza sativa*, showed a stimulating response at 50 Gy giving approximately plant height 19+1.4%; panicle length 27+2.1%; seed number per panicle 64+2.8%, tiller number 17+1.7% while *Phaseolus mungo* showed at 200 Gy giving approximately plant height 51+1.4%; pod length 49+4%; seed number per pod 56+2.8% (Maity et al., 2005). Similarly, the impact of gamma irradiation on maize (*Zea mays*, hybrid Turda Star) seeds was studied, and a radiation sensitivity test was performed to compare germination capacity, plant growth, and photosynthetic pigment contents between treated and untreated seeds. Again, the stimulatory effects of γ-ray were seen at low doses (2–30 Gy) (Marcu et al., 2013). Different examples from literature about gamma irradiation affecting germination characters of different seedlings have been provided in Table 7.

Sound waves based stimulation of seeds

Audible sound within a frequency of 20 Hz to 20 kHz can be heard by human beings. Among different environmental factors like moisture, light, wind, and temperature affecting plant growth, comparatively limited data is available about the effect of audible sounds on plant growth (Hassanien et al., 2014). Acoustic biology has become progressively more popular. Recently different plants have been treated with sound waves to check their effect at various physiological growth stages. The use of sound waves as an invigoration method could reduce the requirement for chemical fertilizers by opening the stomata, and also enhance disease resistance in plants by strengthening the immune system (Junfang, 2012; Carlson, 2013; Jung et al., 2018). Naturally, plants can generate low-frequency sound waves 50–120 Hz and can also absorb or resonate specific frequencies of external sound waves (Frongia et al., 2020). Plants emit also ultrasonic vibrations of 20–100 kHz, measured by connecting a sensor directly to the stem of the plant (Hassanien et al., 2014). Plants release sound emissions from different organs and at different growth stages or in response to different situations. Through the use of small highly sensitive sound receivers, it has been shown that plants emit sound from the xylem and faint ultrasound in case of stress (Jung et al., 2018; Khait et al., 2018). Sound waves can induce various changes such as cell cycle changes, the vibration of plant leaves, and the acceleration of cellular protoplasmic movement (Godbole, 2013). The experimental diagram of sound wave treatment of seed is given in Figure 7.

It has been stated that sound waves activate stress-induced genes and also increase their transcription level (Xiujuan et al., 2003). Sound waves treatment enhances the uptake of dews and sprays fertilizers by stimulating the leaf stomata. Moreover, the process of photosynthesis is also influenced by the conversion of sound energy into chemical energy (Meng et al., 2012). According to previous studies, musical sounds can directly influence the biological system and thus could significantly affect the seeds sprouting (Creath and Schwartz, 2004).

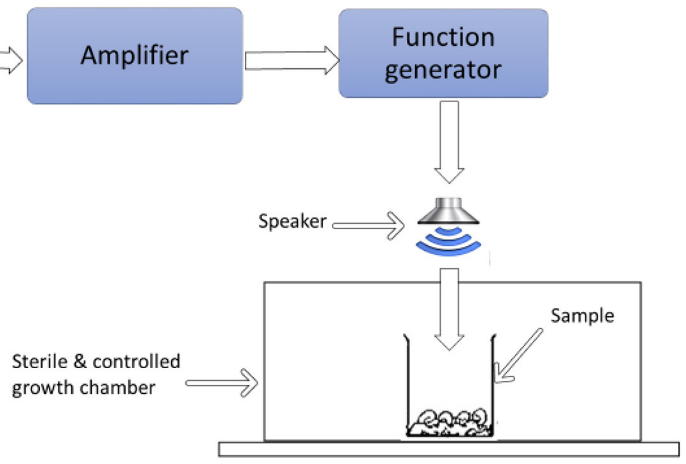

FIGURE 7
Experimental setup of sound wave treatment for seeds.

Several studies have been undertaken to understand the influence of sound and music on plants and plant growth (Table 8). Rideau wheat seeds treated with the sound frequency of 5 kHz and pressure level of 92 dB resulted in stimulated growth along with increased dry weight and number of roots (Weinberger and Measures, 1979). In an experiment, paddy rice seeds were treated with a sound frequency of 0.4–4 kHz and a pressure level of 106–111 dB. The biological effect of sound waves resulted in a significant increase in germination index, stem height, fresh weight, root system activity, and the permeability of the cell membrane (Bochu et al., 2003). According to some studies, music or sound containing hard-core vibrations can cause harmful effects on plant growth. While classical music has gentle vibrations that increase plant growth similarly violin music also induced positive effects significantly (Aladjadjiyan and Kakanakova, 2009). Plants can use acoustic signals from the surrounding environment and spread them rapidly (Gagliano, 2012). A sound wave can transfer energy, to initiate the cytoplasmic streaming and influence the membrane materials thus resulting in variations in biological function and increased metabolic reactions. In another study, at 0.2 and 0.3 kHz, sound frequency young root tips of *Zea mays* showed a clear bend toward a sound source (Gagliano, 2012).

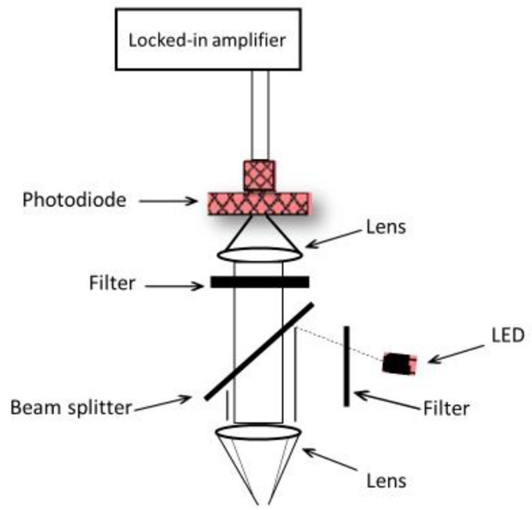

FIGURE 8
Schematic diagram of laser irradiation treatments.

Laser irradiation seed treatment

As in the agriculture field, irradiation is known as a new branch of seed invigoration technique. Laser radiations have features such as coherence, high density, monochromatic, and polarization, and all these properties make laser irradiations applicable in agriculture (Hasan et al., 2020). Various parameters of laser radiation can affect the physiological process in seeds, these parameters include the type of laser radiation, intensity, wavelength, intensity, and exposure time (Govindaraj et al., 2017). The synergistic effect of different mechanisms interacting with laser light results in a range of noticeable effects in the agriculture field. In several studies, laser irradiation showed a positive effect on germination and disease prevention in different crops such as rice, maize, wheat, peas, radishes, and corn (Aladjadjiyan and Kakanakova, 2009; Hernandez et al., 2010). The schematic diagram of the laser irradiation treatment is given in Figure 8.

TABLE 8 The application of sound waves to different crops affects their phenotype.

Plants	Sound frequency	Sound pressure	Effects	References
Rice (*Oryza sativa*)	400 Hz	106 dB	• Increased germination rate, stem height, fresh weight • Improved rooting ability activity of root system and penetrability of cell membrane	Bochu et al., 2003
Rice (*Oryza sativa*)	0.3–6 kHz	80 dB	• Increased growth, yield, and quality	Hassanien et al., 2014
Cowpea (*Vigna unguiculata*)	0.340–3.3 kHz	40–80 dB	• Increased growth and yield	Huang and Jiang, 2011
Wheat (*Triticum aestivum*)	0.340–3.3 kHz	40 dB-80 dB	• Improved seed germination, stem height • Increased activity of root system	Weinberger and Measures, 1979
Mung bean (*Vigna radiate*)	1–1.5 kHz 1.5–2 kHz 2–2.5 kHz	80 dB 90 dB 100 dB	• Increment in stem and root lengths • Reduced germination period with higher sound frequency and sound pressure	Cai et al., 2014

Laser light treatment in seeds induces a series of reactions like accelerated maturity, improved disease resistance, improved energy potential, alpha-amylase action, and free radical's concentration (Klimek-Kopyra et al., 2021). In irradiated seeds, all these reactions lead to reduced seed dormancy, increased rate and percentage of germination, an improved profile of chlorophyll and carotenoid content, higher seed vigor, and a positive effect on the process of respiration and photosynthesis (Wang et al., 2019).

The authors also reported that in addition to serving as a pre-sowing seed treatment, laser irradiations can also affect the quality and quantity of production. Table 9 presents a list of several studies about the effect of laser stimulation on plants that can be found in the literature. In an experiment, He-Ne laser application as a pre-sowing treatment on four spring barley cultivars showed an increment in the germination capacity (Szajsner and Drozd, 2003). Treatment of wheat grains with semiconductor laser influenced their germination and development (Hernandez et al., 2008) while irradiation of tissue culture significantly caused changes in a lipid matrix structure (Salyaev et al., 2007).

The application of light from a laser diode of 650 nm and a power of 27.4 mW increased the germination of photosensitized wheat seeds (Aguilar et al., 2008). Soybean seedlings treated with 532 nm laser improved the photosynthesis proficiency and enhanced the isoflavone content (Tian et al., 2010). Irradiation treatment of *A. farnesiana* seeds for 9 min with He-Ne laser light at 1.70 W cm^{-2} affected the germination indices (Soliman and Harith, 2009). Several other studies used He Ne laser and approved promising effects on germination, in winter wheat genotypes, morphological characters were studied (Szajsner, 2009), in maize hybrids seeds activity of amylolytic enzymes was observed (Podlesny and Stochmal, 2005), and developmental phased of white lupine and fava bean plants were observed (Podlesny and Podlesna, 2004). Similarly, in different experiments, pre-sowing treatment of seeds with irradiation using specific application parameters showed significantly enhanced the production of fava bean seeds (Podlesny, 2007), alfalfa (Dziwulska et al., 2006), wheat (Szajsner, 2009), maize (Szajsner et al., 2007b), and barley (Szajsner et al., 2007a).

Conclusion and future prospects

High vigor seeds represent improved establishment and productivity of crops. Therefore, sustainable crop production requires the use of low-cost and environment-friendly techniques of seed enhancement. Several pre-sowing treatment attempts have been made to improve the yield. Physical methods are an innovation in the research area of seed invigoration to improve crop yield. These physical techniques are the substitute for chemical-based techniques in the development of new biotech-based solutions. These techniques are environmentally friendly and can be used on a high throughput scale. Although, plants respond to the physical treatment but still on a commercial scale it has not been fully exploited. Enough facilities are present to conduct physical treatment of seeds but still, there is a lack of information regarding pre-germinative metabolic reactions occurring in seeds. This information gap is hindering the successful application of these techniques, as seen for chemical treatments. There is also needed to explain all biochemical reactions affecting these processing technologies which result influence the growth and development of plants. These processing techniques have a challenge which is that not all techniques may result in improved germination of seed. The invigoration methods can make the seeds vulnerable to stress conditions if an unsuitable technique is applied to seeds. Therefore, it's important to determine all working conditions and protocols specific to plant seeds. Because the efficiency of

TABLE 9 Effect of laser irradiation stimulation on plants.

Plants	Working conditions	Effects	References
Maize (*Zea mays*), Wheat (*Triticum aestivum*) Barley (*Hordeum vulgare*)	Laser type He and Ne, Power 40–50 mW	• Better plant seedlings • Higher resistance to cold and earlier plant maturation	Koper, 1994
Wheat (*Triticum aestivum*)	Laser type He and Ne	• Effect on morphological characters and yield	Drozd and Szajsner, 1999
Soybean (*Glycine max*)	Laser type He and Ne, Power 7.3 mW	• Reduced the number of seed-borne fungi • Increased germination	Ouf and Abdel-Hady, 1999
Barley (*Hordeum vulgare*)	Laser type He and Ne, Wavelength 632.8 nm, power density-—1 mW cm^{-2}	• Caused stimulation effect on the yield	Rybiński, 2000
Wheat (*Triticum aestivum*)	Laser type As, Al, and Ga	• Stomatal density was diminished • Modified seedling growth and morphology	Benavides et al., 2003
Wheat (*Triticum aestivum*)	Laser type He and Ne	• Increased the strength, germination energy, and seeds respiration	Makarska et al., 2004
Wheat (*Triticum aestivum*)	Laser type He and Ne, Output power 25 mW	• Positive effects on the germination energy	Dinoev, 2006
Maize (*Zea mays*)	Laser type GaAlAs semiconductor, Output power 30 mW, Wavelength 660 nm	• Have significantly increased seed vigor	Hernandez et al., 2006
Wheat (*Triticum aestivum*)	Laser type He and Ne	• Only little effect on growth and grain yield	Wesolowski and Cierpiala, 2006
Wheat (*Triticum aestivum*)	Laser type GaAlAs, Wavelength 850 nm	• Caused bio-stimulated growth	Hernandez et al., 2008
Maize (*Zea mays*)	Laser type Diode, Output power 27.4 mW, Wavelength 650 nm	• Negatively bio stimulated the seedling emergence % and emergence rate	Hernandez-Aguilar et al., 2009

each technique is directly linked to different factors including plant species, cultivars, environmental conditions, type of technique, processing treatment dose, exposure timings, etc. There is also needed to expand the number of tested species with each technique to identify factors best appropriate for each physical treatment. Further, for each treatment to modulate the seed response, the study of environmental parameters and their impact could not be ignored.

Author contributions

SH and AR designed the study and wrote the manuscript's first draft. MK and MF conducted sample selection and data management. Mahwish, TT, and AK managed the literature searches and analyses. X-AZ and SH edited the manuscript and supervised the work. AA and AL contributed in writing-review and editing the final manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of interest

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

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Impact of different processing techniques on reduction in oil content in deep-fried donuts when using kombucha cellulose hydrolysates

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To evaluate the efficiency of the oil-reducing properties of kombucha cellulose, enzymolysis and microwave-assisted enzymolysis methods were developed. The water-holding capacity of the kombucha cellulose hydrolysates formed by these two methods was higher than for the intact kombucha cellulose, while the oil-holding capacity was lower. The hydrolysates of kombucha cellulose and the intact kombucha cellulose were used to make deep-fried donuts. During this process, kombucha cellulose hydrolysates were added instead of 2% flour, and from the results, the oil content of the donut decreased significantly from ∼28 to 15%, and the reduction was not related to the processing of the donut. The hardness and brittleness of all samples showed no significant change, and these samples had similar internal micro-structures, confirming texture profile analysis. *In vitro* digestion results suggested that there would be no adverse health effects from substituting kombucha cellulose hydrolysates in the deep-fried donut formula.

KEYWORDS

kombucha cellulose hydrolysates, deep-fried donut, oil content reduction, texture profile, *in vitro* digestion

Introduction

Kombucha is a functional fermented tea with a sweet and sour taste. This kind of beverage usually contains sugars, polyphenols, organic food acids, fibers, ethanol, amino acids, vitamins, and essential minerals (Kapp and Sumner, 2018). It is a symbiotic colony of yeasts (mainly *Acetobacter xylinoides* and *Acetobacter ketogenum*) and bacteria (Tran et al., 2020). It was first produced in the northeast of China around 220 BC, and in recent years, it has gained tremendous popularity in Europe and America (Kim and Adhikari, 2020). Kombucha is a functional beverage, which has shown the fastest growth in the markets due to its beneficial effect on human health (Kim and Adhikari, 2020). There has been an increase of up to 30% annually to kombucha retail sales worldwide since 2017, and over the next few years, the market in the United States is expected to expand at a pace of 17.5%. During fermentation, a by-product, kombucha cellulose hydrolysates, is formed by acetic acid bacteria as a cellulose biological film in the liquid–air interface. Unlike the celluloses derived from plants, it shows excellent mechanical strength, a high degree of water absorption, high purity, and a high degree of polymerization, and it does not contain hemicellulose or lignin (Oliveira et al., 2017). Kombucha cellulose was first regarded as a waste product in the process of fermentation, although this biomaterial has recently been applied to different fields due to its diverse physical and chemical properties. For example, it has been used in forming hydrogel cellulose (Esa et al., 2014) and in the pyrolytic generation of graphene oxide (Amarasekara and Wang, 2021). It has also been shown to reduce *Escherichia coli* in the dairy shed effluent (Laurenson et al., 2021) and is used to fabricate electrolyte membranes (Vilela et al., 2018). However, in these previous studies, structural modifications of the cellulose have been chemically derived, and there has been no investigation of kombucha cellulose as a functional food.

Deep frying is a quick and suitable food preparation process. It provides distinctive sensory properties, making the food crunchy on the outside, with a soft and moist interior that customers from different countries love. There are many typical deep-fried desserts in Western countries, and the deep-fried donut is prominent among them. Customers favor the fried donut due to its unique flavor, crispy texture, and sweet taste (Saguy and Dana, 2003).

Because there is a great amount of oil in deep-fried food products, it is considered unhealthy. Studies have found that an excessive oil intake can cause hypertension and obesity (Peter et al., 2018). The oil content of raw fish can be as much as 190 g/kg, up from approximately 20 g/kg after frying (Barbara et al., 2016), while the oil content of deep-fried chips can reach approximately 400 g/kg (Kita and Golubowska, 2007). In general, after deep frying, the oil content of food can reach up to one-third of its overall weight (Mia et al., 2017). According to research, the oil level in deep-fried food products may be successfully lowered by using specific substances in the batter formula. For instance, adding 10 ml/L methylcelluloses and 5 ml/L sorbitol into the batter of deep-fried potatoes can significantly decrease its oil content by 40.6% (Bo et al., 2017). Another study increased the proportion of wheat flour from 1 to 5% in soybean hull-coated frying batter, and the oil content of the final deep-fried food was reduced from 244 to 33 g/kg (Oke et al., 2018). However, few studies have been reported on decreasing the oil content in deep-fried foods and the effects of water- and oil-holding capacities of FPH on the oil content of deep-fried foods.

In this study, cellulose from kombucha was applied as a sample. This study aims to analyze the several preparation procedures for the water-holding and oil-holding capabilities of hydrolysates made from kombucha cellulose. Furthermore, we report the impacts of kombucha cellulose hydrolysates on the reduction of oil content and the texture profiles for deep-fried foods.

Materials and methods

Materials

Constituents bought from a local market were used to prepare the kombucha. First, a sugared liquid solution was prepared by adding 100 g of sucrose in 800 ml of distilled water and heated for 15 min at 98°C. At first, 4 g green tea was soaked in the sugared liquid for 12 min and then filtered to remove leaves. The mixture's temperature was reduced to 25°C, then inoculated with 600 ml kombucha (Shenzhen Care Pack Co. Ltd.) to begin fermentation. The cellulose film for kombucha was produced after a 15-day fermentation of the culture.

Kombucha cellulose purification

The film was dipped in distilled water for 2 days. Filtration of kombucha was performed by the Buchner funnel to eliminate bacteria and impurities from the mixture. Then, the filtered sample was treated with 1 M NaOH at 50°C for 12 h, which was then neutralized for 1 h by using 1% glacial acetic acid. A neutral pH of washing water was achieved by washing the kombucha cellulose in distilled water and freeze-dried it for further use.

Kombucha cellulose hydrolysates produced using the enzymatic treatment and microwave-intensified enzymatic treatment

Commercial cellulase was used to perform the enzymatic treatment. The cellulase enzyme activity in the kombucha–cellulose mixture with a concentration of 10% (w/v) was 300 leucine aminopeptidase units per gram (LAPU/g). One aminopeptidase unit per gram (LAPU/g) is the amount of enzyme that hydrolyzes 1 μmol of leucine-*p*-nitroanilide in 60 s. To reach these optimal conditions, the kombucha–cellulose mixture temperature was set to 50°C and the pH was set to 7–8 (adjusted to a neutral pH in 2.2). A water bath was used to perform a 1-h enzymatic treatment with an enzyme:substrate ratio of 1.0% (w/w). A microwave synthesis laboratory station (Milestone S.r.L, Sorisole (BG), Italy) was used to perform the microwave-intensified enzymatic treatment at 500 W. The enzyme:substrate (E:S) ratio was set at 1.0% (w/w) for 10 min.

Following each process, enzyme activity was restricted by boiling at 100°C for 10 min. The processed hydrolyzed solution was centrifuged at 4,000 × g for 30 min, resulting in two layers: an unhydrolyzed, insoluble cellulose precipitate layer at the bottom and a hydrolyzed, soluble cellulose hydrolysate liquid layer on the top. The top layer was collected and freeze-dried. Cellulose hydrolysate powder was stored in sealed 50 ml tubes in a desiccator at room temperature.

Determination of oil-holding capacity

Determination of the oil-holding capabilities was done with the method by Zhang et al. (2015). In brief, the 1 g sample was weighed in a centrifuge tube. A total of 9 g of canola oil was added and well mixed using a vortex mixer and subsequently allowed to stand at room temperature. The oil-holding capacity was recorded by calculating the weight of the oil that was absorbed by the 1 g sample, compared with the original 9 g of canola oil.

Determination of water-holding capacity test

Bowker and Zhuang (2015) method, with a few modifications, was used to determine the water-holding capacity. A total of 1 g of sample was mixed with 5 g of ground fresh carp fish mince, and then added 8 g of tap water. Further, the sample was incubated at 50°C for 1 h, followed by centrifugation at 340 × g for 10 min, and the weight of the supernatant was recorded. The weight difference between the supernatant and added water measured the water-holding capacity.

Particle size distribution of kombucha cellulose and kombucha cellulose hydrolysates

Dynamic light scattering (DLS) (Nano ZS90, Malvern instruments, Worcester, UK), was applied to measure the particle size distribution. The freeze-dried samples were crushed and dispersed in water with a concentration of 2% (w/v) for DLS studies. Based on the particle sizes, the time-dependent fluctuations of light scattered were measured using a Malvern zeta-sizer instrument.

Deep-fried donut production

The dough and donut were prepared by the proposed method of Sirichokworrakita et al. (2015) with slight modifications. To prepare the control sample, 3 g of yeast was weighed, and 10 ml of water was added and incubated for 20 min. Further, the dough's control sample was prepared by adding 40 g of sugar, 2 g of salt, and 3 g of yeast to the 100 g of flour and mixed until the dough base was fully developed. As for the other samples, 2 g of 100 g flour was replaced with kombucha cellulose hydrolysates or kombucha cellulose. In a pan, 60 ml of milk and 15 g of butter were heated. Melted butter was added to the dough mixer. Then, two drops of vanilla extract and a whole egg (110 g) were added in a mixing pan followed by adding the sunflower oil. The dough was covered with a bowl cover and incubated for 2 h at room temperature. Once the dough volume increased, it was placed on a clean flat surface. The 3 cm diameter round donut cutter was used to shape the donuts after being rolled to a thickness of 1.27 cm. Then, donuts were deep fat fried into 2 L of sunflower oil (purchased from a local market) approximately for 4 min at 150°C (Zolfaghari et al., 2013; Arash et al., 2018). Furthermore, we took out the donuts from the frying pan and drained any remaining oil using absorbent paper. The processed donuts were cooled at room temperature, placed in high-density polyethylene, and kept for further analysis.

Measurement of texture profile analysis

Texture profile analysis (TPA) was used to measure deep-fried donuts' hardness (g) and brittleness (mm). After deep frying, the donuts were cut immediately into a cylindrical form. The height of the cut samples was 2 cm, and the diameter

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was 2.5 cm. Further, the texture of the samples was analyzed by putting them in a specimen of texture analyzer, Perten Instruments 31, SE-126, 53, Hagersten, Sweden) and analysis was performed with a speed of 2.0, 1.0, and 2.0 mm/s, for pre-test, test, and post test, respectively; and 50% sample deformation. After each compression, the force deformation graph was recorded by the analyzer to calculate the hardness and brittleness of the samples (Figure 1).

Determination of donut internal micro-structure

The donut's internal structure was measured with a scanning electron microscope (SEM) (Alto 2100, Gatan Ltd., Abingdon, UK), and by the method followed by Vos et al. The colonial-shaped samples were cut by a Stanley knife with a diameter of 1.5 mm × 1.5 mm, and samples adhered to gold stage. Further, the samples were frozen in liquid nitrogen. In the sample preparation room, the head of the specimen shattered, and furthermore, the sublimation of samples was done at −90°C. The internal structure of the samples was analyzed at 30 kV with 5.0 spot values to see and evaluate the interior micro-structure.

Determination of water and oil contents

The water and oil content was determined with the ethylene to deaminate AOAC procedures 950.46 and 960.3928, respectively.

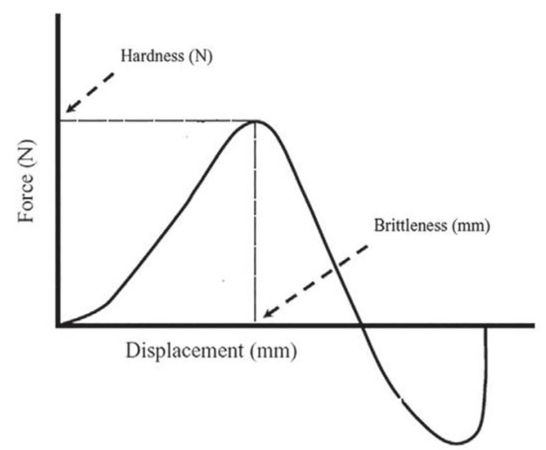

FIGURE 1
Instrumental texture analysis yielded a force–displacement curve that displayed hardness and brittleness.

In vitro digestion

The *in vitro* digestibility of the sample (donut) was determined with a method by Gao et al. (2016). In brief, the 2.5 g of doughnut and 30 ml of distilled water were mixed in a biopsy pot. Further, we put in a heated magnetic stirring block (IKAAG RT 15, IKA-Werke GmbH & Co., Staufen, Germany), and after 10 min of stirring at 37 °C, we prepared the solution with 0.8 ml HCL (1 M) and 1 ml pepsin (10%) and added in 0.05 M HCl to imitate the stomach digestion. Then we stopped the digestion process after 30 min using 2 ml NaHCO3 (1 M). Therefore, 5 ml sodium maleate (0.1M) buffer having pH 6, and 5 ml of 2.5% pancreatin solution in 0.1 M sodium maleate buffer with pH 6 were added to start small intestine digestion, which was continuously stirred for ∼120 min. Moreover, we took 1 ml aliquots before the addition of the pancreatin and added them to 4 ml ethanol at different time intervals such as 20, 60, and 120 min. To analyze reducing sugar content [3,5-dinitrosalicylic acid (DNS) method], samples were kept at 4°C. Allotted into trapezoids, the graph area under the curve (AUC) was determined. After being freeze-dried, the digest supernatants were put in storage at 20°C.

Statistical analysis

After three replications of each measurement, the data were presented as means with standard deviations. To conduct the statistical analysis, Minitab was used to apply a one-way analysis of variance (ANOVA) and the least significant difference (LSD). The F value at a probability (p) $\leq$ 0.05 was used statistically to determine the significance.

Results and discussion

The oil-holding and water-holding capacities of kombucha cellulose and kombucha cellulose hydrolysates

The oil-holding capacities are shown in Table 1. Any liquid oil can be used in the measurement of oil-holding capacity with the same principle: The oil-holding capacity was recorded by calculating the weight of the oil that was absorbed by 1 g of the sample, compared with the original weight of the oil. Considering that canola oil is one of the cooking oils that is the easiest to be sourced in the market, and many previous studies used canola oil for oil-holding capacity measurement (Li et al., 2020; Hadi and Sudiyono, 2021), this study also selected canola oil for the measurement of oil-holding capacity.

The analysis stated that the oil-holding capacities of kombucha cellulose hydrolysates were significantly reduced as

TABLE 1 Oil-holding and water-holding capacities of samples from various processing techniques.

S. No.	Processes	Oil-holding capacity (g/g)	Water-holding capacity (g /g)
1	Kombucha cellulose	$16.56^{a} \pm 0.78$	$11.04^{a} \pm 0.77$
2	Kombucha cellulose by enzymatic treatment	$6.11^{b} \pm 0.23$	$18.23^{b} \pm 0.95$
3	Kombucha cellulose by microwave-intensified treatment	$2.18^{c} \pm 0.84$	$19.32^{b} \pm 1.09$

Probability (p) ≤ 0.05 was used statistically to determine the significance. $^{*}\pm$ SD is the mean of three measurements per trial.
Within each trial, different letters indicate significant differences ($p < 0.05$), according to one-way ANOVA and LSD test.

compared to the original kombucha cellulose (16.56 g oil/g). The lowest oil-holding capacity was shown with kombucha cellulose hydrolysates produced by microwave-intensified enzymatic treatment (2.18 g oil/g sample), which is a reduction ratio of ∼88%. It has been reported that the size reduction of biomaterials may cause the reduction of its oil-holding capacities. For example, He et al. (2012) reported that the oil-holding capacity dropped from its highest capacity of 14.77 g oil/g sample to the lowest capacity of 4.45 g oil/g in fish protein after its molecular weight was reduced from above 100 kDa to 0–30 kDa *via* enzymatic hydrolyzation, which is a reduction ratio of ∼71%. The oil-holding capacity of the kombucha cellulose hydrolysates that were produced *via* microwave-intensified enzymatic treatment was significantly lower than other samples (2.18 g/g in comparison with 6.11 and 16.56 g/g). The DLS measurement demonstrated a substantial reduction in particle size from kombucha cellulose (Kashcheyeva et al., 2019) to kombucha cellulose hydrolysates produced from microwave-intensified enzymatic treatment (Kashcheyeva et al., 2019) (Figure 2), which is consistent with previous findings. However, as observed by SEM (Figure 3), the kombucha cellulose showed network micro-structures cross-linked by multiple long chains of intact cellulose fibers. By contrast, after hydrolyzation, the cross-linked network micro-structure was dismantled due to the multiple long chains of intact cellulose fibers being broken down into tiny pieces through hydrolyzation and therefore unable to form a network. The better the cross-linked network of microstructures in the bio-materials, the more oil it could entrap in this micro-structure, increasing its oil-holding capacity (Tanti et al., 2016). This combinatorial effect of a reduction in particle size and dismantling of the cross-linked network micro-structure caused by enzymatic hydrolyzation explains the higher reduction ratio of the oil-holding capacity.

The kombucha cellulose hydrolysates showed a higher water-holding capacity than the kombucha cellulose. It has been reported that kombucha cellulose has a high level of hydrophilicity (Guzel and Akpinar, 2020). This refers to abundant hydrophilic groups of kombucha cellulose that are exposed to the surroundings. The increased hydrophilic groups during the hydrolyzation process are the reason for the improved water-holding ability of kombucha cellulose. Furthermore, a higher frequency of exposed hydrophilic groups led to a lower frequency of exposed hydrophobic groups, and after hydrolyzation, it also contributes to the reduction of oil-holding capacity.

It is noteworthy that though the microwave-intensified enzymatic processing period (10 min) was significantly shorter than the time taken for enzymatic treatment (1 h), it was more efficient in terms of hydrolyzation, as indicated by the smaller particle size and the oil-binding capacity of the kombucha cellulose hydrolysates produced. The heat is transmitted from the outside, which caused delays with traditional heating techniques (such as heating water baths). This delay was quickly reduced by the electromagnetic energy heating of microwave irradiation (Thostenson and Chou, 1999). Microwaves heat evenly from the inside samples (Su et al., 2022).

Donuts water and oil contents

Table 3 lists the water and oil content of deep-fried donuts processed using different preparation methods. The oil content of the donuts processed with kombucha cellulose hydrolysates was ∼15.32%, while control samples had an oil content of 28.11%. There was no discernible change between the donut oil content of the control sample and that of the sample with the addition of the original kombucha cellulose. The oil-holding capacity is very important in food products, and ingredients with different oil-holding capacities have been studied and employed to control the oil content and structure of food products (Rajan et al., 2014). It is reasonable to believe that the oil contents of deep-fried donuts with FPH from various treatments would be positively correlated with the oil-holding capacities of samples from different treatments. However, this correlation could not be established due to the model of the deep-fried food products. First, while frying, the donut was not evenly in contact with the oil; moreover, the major inner part of the donut was not in contact with the oil. The mechanism for the volume ratio of the outside (crust) to the inner part of deep-fried foods has been studied by (Kumar et al., 2014), and the oil does not penetrate deeply into the interior during deep frying. The thickness of the food crust during deep frying is ∼6 mm, while the oil in the outside crust can be 6–10 times greater than that which is

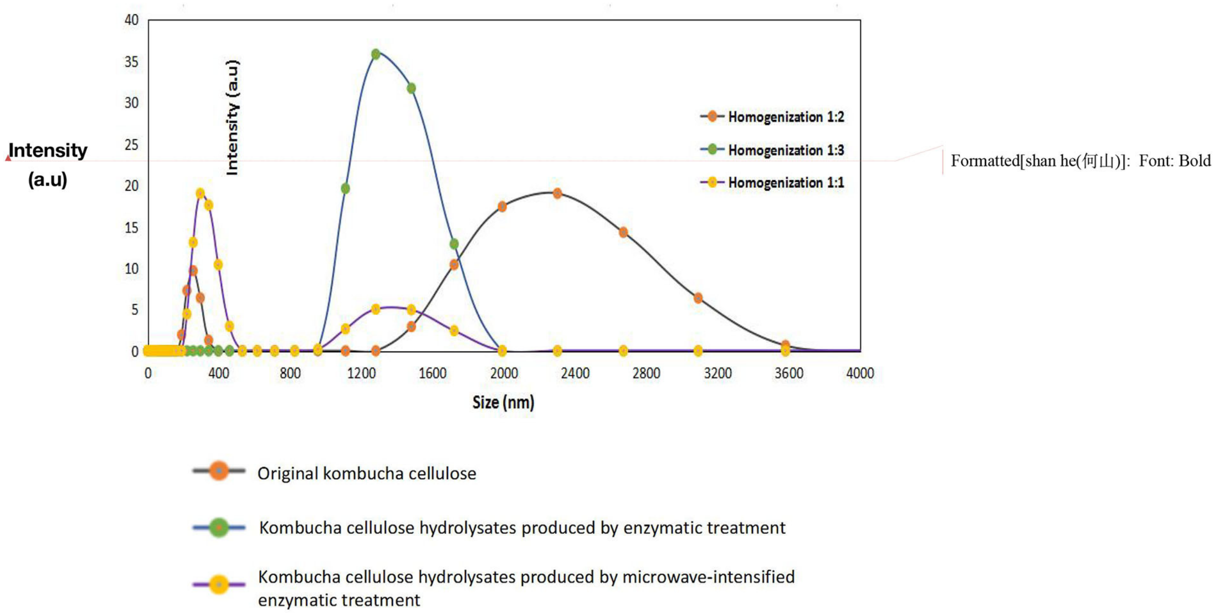

FIGURE 2
DLS data at 25°C (He–Ne laser 633 nm; detector angle 173°) for original kombucha cellulose, kombucha cellulose hydrolysates produced *via* enzymatic treatment, and kombucha cellulose hydrolysates produced using microwave-intensified enzymatic treatment.

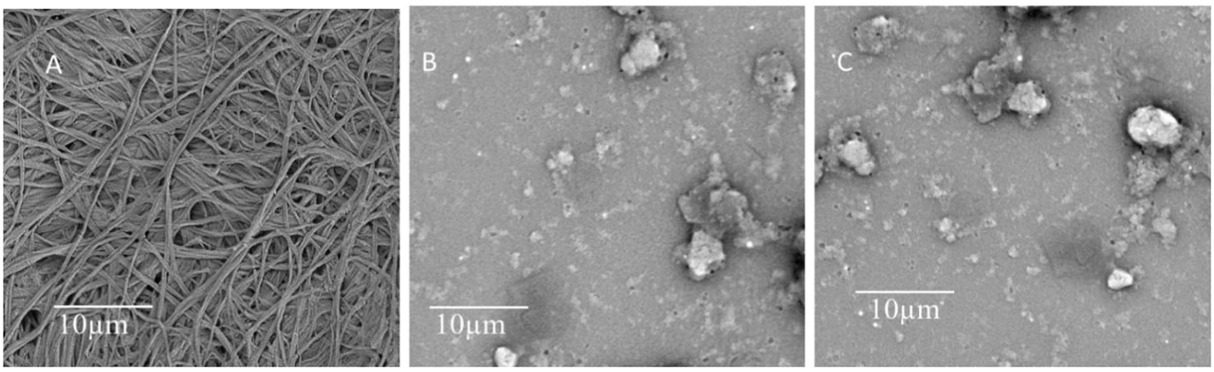

FIGURE 3
The internal images of **(A)** original kombucha cellulose. **(B)** Kombucha cellulose hydrolysates produced *via* enzymatic treatment. **(C)** Kombucha cellulose hydrolysates produced *via* microwave-intensified enzymatic treatment.

absorbed by the inner parts. On average, there may be no discernible variation in the oil content of the complete deep-fried food product.

However, Table 3 indicated a positive correlation between the oil content in the donut crust and the oil-holding capacity of the samples. It is also noticeable that the oil content in the crust was much higher than that in the entire donut after deep frying (Table 3), while a lower water content was observed in the whole donut (Table 2).

The oil content of the deep-fried donuts (Table 1) and their water-holding capabilities (Table 2) were correlated. The samples' ability to retain water increases as oil concentration decreases. Because of the higher water-holding capacity, the water content after deep frying was higher. The oil content of fried food is affected by its water content. An increase in oil content is related to a reduction in water content. After the water is removed from fried food, oil can easily penetrate into it. The temperature of the food surface rapidly rises with the addition of heated oil, resulting in immediate boiling of the water on the food surface (Giuseppe and Camilla, 2015). This leads to low water content and surface porosity on the crust of the deep-fried food (Table 3). The crust structure has many

TABLE 2 Water and oil content of deep-fried donuts produced using various techniques.

S. No.	Processes	Water content (%)	Oil content (%)
1	Kombucha cellulose	$23.40^{a} \pm 0.72$	$28.11^{a} \pm 0.33$
2	Kombucha cellulose *via* enzymatic treatment	$29.05^{b} \pm 0.38$	$15.32^{b} \pm 0.38$
3	Kombucha cellulose *via* microwave-intensified treatment	$30.81^{c} \pm 0.41$	$14.92^{b} \pm 0.12$
4	Control (deep-fried donuts without cellulose)	$23.11^{a} \pm 0.50$	$28.25^{a} \pm 0.03$

Probability (p) ≤ 0.05 was used statistically to determine the significance. $^{*} \pm$ SD is the mean of three measurements per trial.
Within each trial, different letters indicate significant differences ($p < 0.05$), according to one-way ANOVA and LSD test.

TABLE 3 Water content and oil content of deep-fried donut crusts made using different processes.

S. No.	Processes	Water content (%)	Oil content (%)
1	Kombucha cellulose	$1.45^{a} \pm 0.31$a	$67.30^{a} \pm 2.03$
2	Kombucha cellulose *via* enzymatic treatment	$1.48^{a} \pm 0.54$a	$57.30^{b} \pm 2.03$
3	Kombucha cellulose *via* microwave-intensified treatment	$1.76^{a} \pm 0.36$a	$36.63^{c} \pm 0.22$
4	Control (deep-fried donuts without cellulose)	$1.24^{a} \pm 0.18$a	$25.86^{c} \pm 0.45$

Probability (p) ≤ 0.05 was used statistically to determine the significance. $^{*} \pm$ SD is the mean of three measurements per trial.
Within each trial, different letters indicate significant differences ($p < 0.05$), according to one-way ANOVA and LSD test.

TABLE 4 Brittleness* and hardness* of deep-fried donuts made using various techniques.

S. No.	Processes	Brittleness (mm)	Hardness (N)
1	Kombucha cellulose	$12.12^{a} \pm 0.51$	$18.87^{a} \pm 2.65$
2	Kombucha cellulose *via* enzymatic treatment	$13.88^{a} \pm 3.60$	$17.21^{a} \pm 1.73$
3	Kombucha cellulose *via* microwave-intensified treatment	$11.76^{a} \pm 1.71$	$17.31^{a} \pm 1.39$
4	Control (deep-fried donuts without cellulose)	$10.83^{a} \pm 2.63$	$16.88^{a} \pm 2.03$

Probability (p) ≤ 0.05 was used statistically to determine the significance. $^{*} \pm$ SD is the mean of three measurements per trial.
Within each trial, different letters indicate significant differences ($p < 0.05$), according to one-way ANOVA and LSD test.

voids for oil to penetrate the interior. Free water is more easily evaporated than bound water. Kombucha cellulose hydrolysates have a strong water-holding capacity. The water inside the donut evaporates more slowly, lowering the number of holes for oil to enter, resulting in low oil content.

Texture analysis of donuts

A texture analyzer was used to measure the influences of water and oil content on the texture of deep-fried donuts. While testing, the samples were compressed two times to mimic chewing behavior by a human. Amada et al. subjected different extruded snacks to TPA and a sensory test (Di et al., 2014) and analyzed the adhesiveness, hardness, fracturability, crispness, and chewiness of extruded snacks, although different snack food shapes could also cause different sensory profiles. The two most crucial parameters in the TPA test for foods produced using flour were hardness and brittleness (Amanda and Ana, 2014). Table 4 shows the hardness and brittleness of kombucha cellulose, kombucha cellulose treated with enzyme and microwave, and deep-fried kombucha cellulose without cellulose as a control sample. The results showed that the brittleness of kombucha cellulose treated by an enzyme (13.88 ± 3.60) and microwave (11.76 ± 1.71) increased as compared to control sample (10.83 ± 2.63). while it could be noticed that brittleness of microwave-assisted kombucha cellulose decreased as compared to enzymatic treatment. Further analysis of the hardness of these samples revealed that enzymatic- (17.21 ± 1.73) and microwave (17.31 ± 1.39)-treated kombucha cellulose exhibits more hardness compared to the control sample (16.88 ± 2.03). The characteristics of deep-fried donuts manufactured using various procedures, and these two parameters revealed no significant changes across samples, although the water and oil content of the deep-fried donuts made using various methods differed (Table 2). Deep-fried food products' textures are impacted mainly by their soft and wet core, while the exterior crust offers sensory qualities, that is, color and flavor. It also shows that their impact on the water lost during the deep-frying process is primarily free water (Huanghuang et al., 2017).

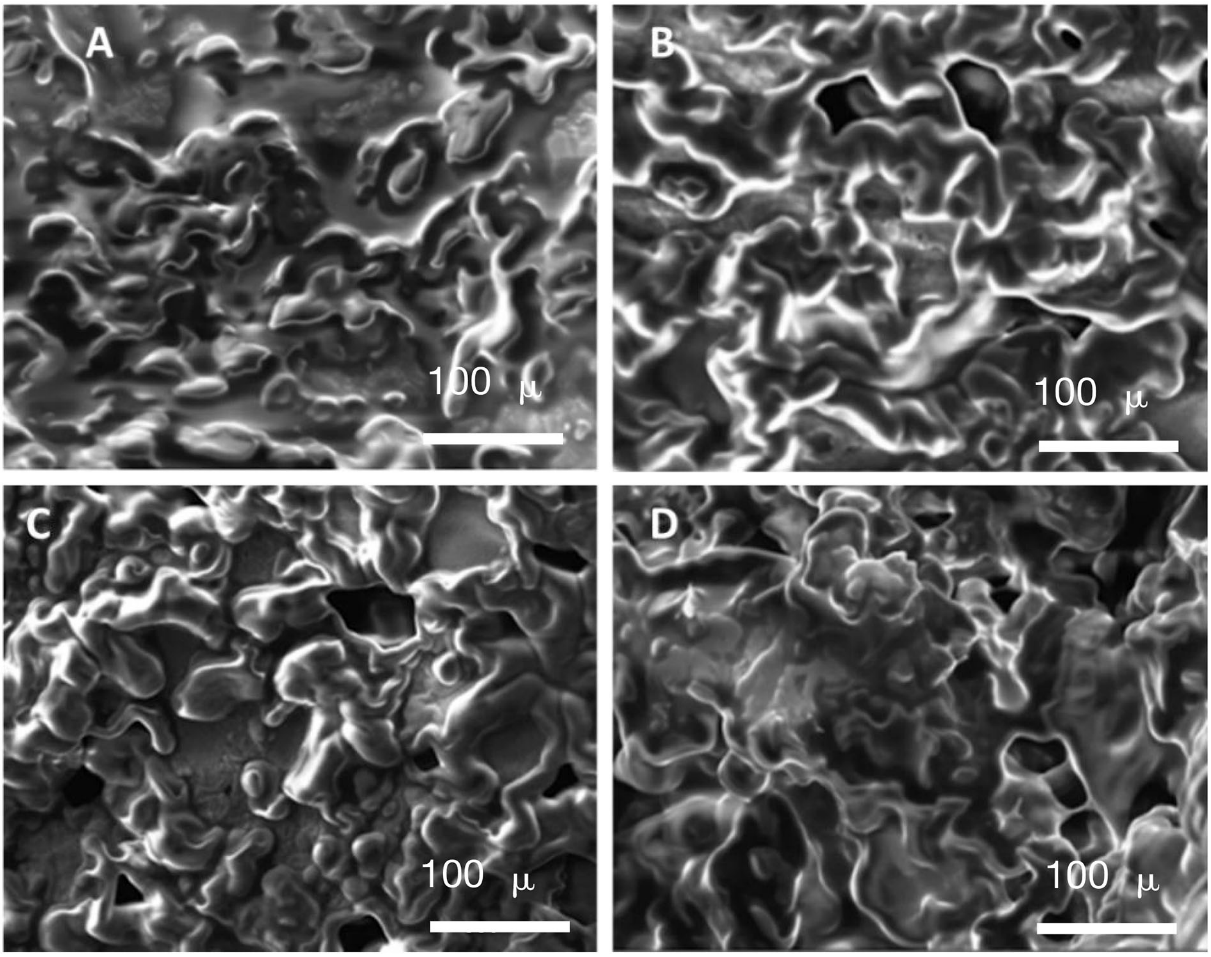

FIGURE 4
The micro-structures of deep-fried donuts are produced using different processing methods. **(A)** Kombucha cellulose, **(B)** Kombucha cellulose produced *via* enzymatic treatment, **(C)** Kombucha cellulose produced *via* microwave-intensified treatment, and **(D)** Control (deep-fried donuts without cellulose) using a scanning electron microscope (×3,000).

However, there was no more water loss from the control sample (with no additional ingredients) when compared with the deep-fried donut produced with the addition of kombucha cellulose or cellulose hydrolysates. According to (Koerten et al., 2017), a significant shift in the free water percentage from 50 to 30% had little impact on the food items' textural characteristics, and they demonstrated how the mechanically trapped water primarily influenced the food textural attributes.

Micro-structures of donuts

Scanning electron microscopy is a dependable method for exploring the inner micro-structures of matrices in food products (Koerten et al., 2017). The texture profile of the food can be determined by using structured matrices (Wilkinson et al., 2000). Shan et al. (2010) found that steam-cooked kamaboko with a pre-treatment of carp mince washed with aqueous H_2O_2 under optimal conditions possessed the highest degree of chewiness and hardness because of its more regular, cross-linked inner micro-structure. Figure 4 shows the images of deep-fried donuts produced using different processes. There were no significant inner structural differences, indicating the similarity of textures in deep-fried donuts produced using different methods. This finding was in accordance with the relationship between the texture of deep-fried kamaboko and its inner structure reported by Zeng et al. (2018).

In vitro digestion

The amount of reducing sugar released by *in vitro* digestion of deep-fried donuts is shown in Figure 5. The risk of obesity and

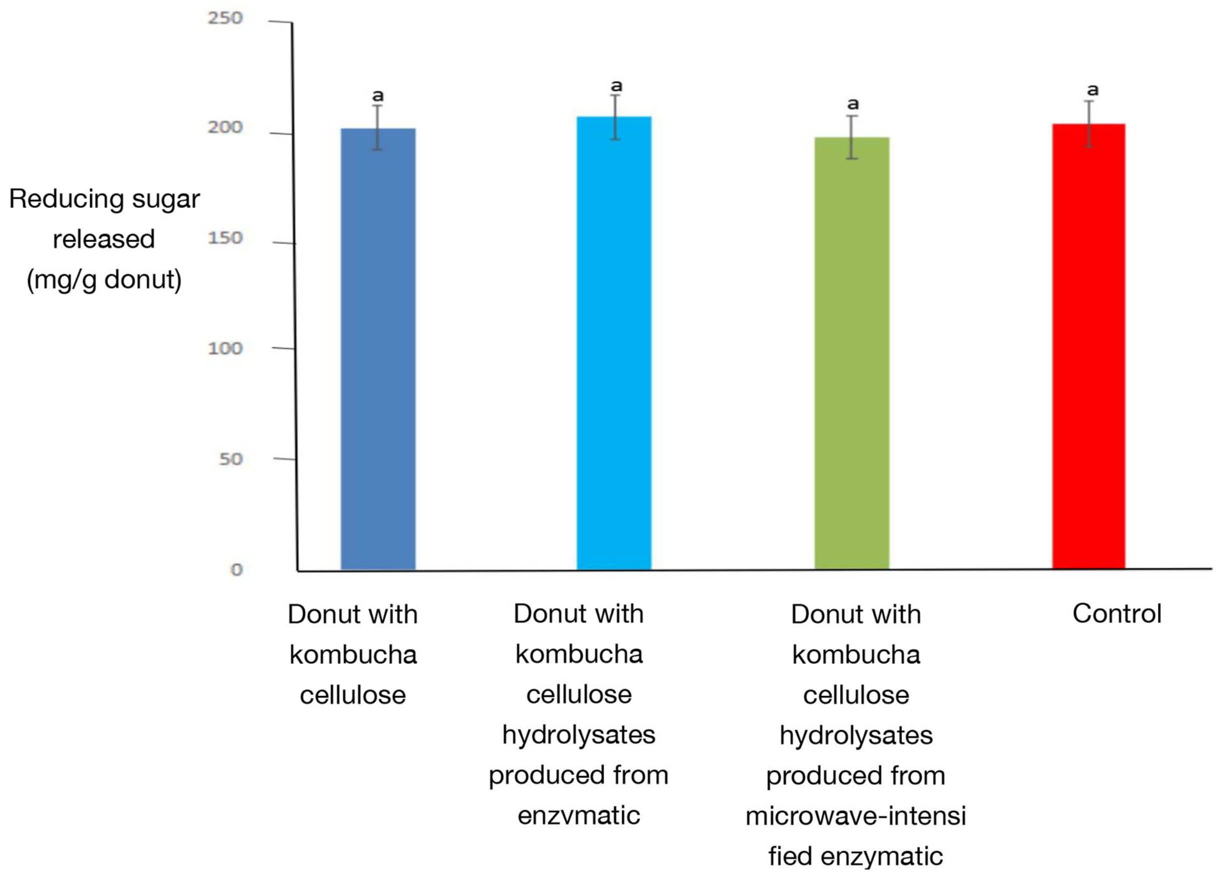

FIGURE 5
The AUC of deep-fried donuts produced by different treatments. Error bars represent the standard deviation of replicates ($n = 3$). Values with different letters indicate significant differences ($p < 0.05$). Minitab was used to apply a one-way analysis of variance (ANOVA) and the least significant difference (LSD).Within each trial, different letters indicate significant differences ($p < 0.05$), according to one-way ANOVA and LSD test.

dental decay can be decreased by reducing the intake of sugar (Stanhope, 2016). The results of this *in vitro* digestion show that the release of reducing sugars was unaffected by the different treatments. This is different from protein-based materials. Xi et al. (2021) reported that in comparison with cereal protein, cereal protein hydrolysate increased the release of reducing sugars when it was applied as a food supplement in bakery products. However, the cellulose-based materials presented in this study do not show this negative health impact.

Conclusions

Kombucha cellulose hydrolysates were developed by enzymatic treatment and a microwave-assisted enzymatic treatment as well. The water-holding and oil-holding capacities of kombucha cellulose hydrolysates created from both processes showed all higher and lower capacities, respectively, compared to intact kombucha cellulose. By replacing flour with 2% kombucha cellulose hydrolysates in the production of deep-fried donuts, the oil contents of the deep-fried donuts could be significantly decreased from ∼28 to 15%. The texture profile analysis results showed slight differences, that is increasing brittleness and hardness, as compared to the deep-fried donut samples without cellulose. SEM analysis found that the deep-fried samples' inner microstructures were also similar or exhibit negligible differences. *In vitro* digestion test did not change the release of reducing sugars. So, this study concludes that kombucha cellulose hydrolysates in deep-fried donuts would not show adverse health effects.

Data availability statement

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

Author contributions

SH, YZ, JG, and CG designed the study protocol and wrote the manuscript's first draft. YW, MF, and DY conducted sample selection and data management. JW and KC managed the literature searches and analysis. BT, AK, and AR edited the manuscript. SH supervised the work. All authors contributed to and have approved the final manuscript.

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Conflict of interest

Authors SH and KC shared an affiliation with Peats Soil and Garden Supplies.

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Evaluation of the antimicrobial effects of *Capsicum, Nigella sativa, Musa paradisiaca* L., and *Citrus limetta*: A review

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The extensive use of antibiotics and vaccines against microbial infections can result in long-term negative effects on humans and the environment. However, there are a number of plants that have antimicrobial effects against various disease-causing microbes such as bacteria, viruses, and fungi without negative side effects or harm to the environment. In this regard, four particular plants- *Capsicum*, *Nigella sativa*, *Musa paradisiaca* L., and *Citrus limetta* have been widely considered due to their excellent antimicrobial effect and ample availability. In this review, we discuss their antimicrobial effects due to the presence of thymoquinone, p-cymene, pinene, alkaloids, limonene, camphene, and melanin. These antimicrobial compounds disrupt the cell membrane of microbes, inhibit cellular division, and form biofilm in bacterial species, eventually reducing the number of microbes. Extraction of these compounds from the respective plants is carried out by different methods such as soxhlet, hydro-distillation, liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), solid-phase extraction (SPE), supercritical fluid extraction (SFE), pulsed electric field (PEF), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), and high-voltage electrical discharge. Suitable selection of the extraction technique highly depends upon the associated advantages and disadvantages. In order to aid future study in this field, this review paper summarizes the advantages and disadvantages of each of these approaches. Additionally, the discussion covers how antimicrobial agents destroy harmful bacteria. Thus, this review offers in-depth knowledge to researchers on the antibacterial properties of *Capsicum, Nigella sativa, Musa paradisiaca* L. peels, and *Citrus limetta*.

KEYWORDS

antimicrobial agent, antimicrobial mechanism, solid waste management, extraction techniques, *Capsicum*, *Nigella sativa*, *Musa paradisiaca* L., *Citrus limetta*

Introduction

A global public health crisis has been caused by the rise in resistant bacteria over the past few decades (Dhingra et al., 2020; Hu et al., 2020; Ibrahim et al., 2021). Although antibiotics are used to treat illnesses by killing bacteria or limiting their growth, they can also have several negative effects (Gyawali and Ibrahim, 2014). For example, because some disease-causing bacteria have acquired resistance to particular antibiotics as a result of their widespread and sustained usage, these treatments are no longer as effective (Namita and Mukesh, 2012). In this regard, antimicrobial agents can eliminate resistant pathogenic microbes without imposing any known side effects. Antimicrobial agents disrupt the cellular structure of microbes, thus inhibiting various infectious diseases (Peterson, 2008) and are used to treat humans with diseases that are primarily due to non-human sources (Organization, 2019). According to WHO (world health organization), the overall consumption of antimicrobial agents is in between 4.4 and 64.4 Defined Daily Doses (DDD) per 1,000 persons per day. As per WHO data, the national estimates of antimicrobial consumption in 65 countries and areas: six from the Region of America, four from the African Region, three from the Eastern Mediterranean Region, 46 from the European Region: and six from the Western Pacific Region are presented in Figure 1 (Organization, 2018).

According to WHO, residents of these low-income countries are more likely to die of communicable and infectious diseases such as malaria, tuberculosis, diarrhea, HIV/AIDS, etc. (Philip, 2017). Therefore, suitable preventive measures against these infectious and parasitic diseases should be adopted. These measures may include self-cleanliness, proper sanitation, safe food preparation and handling, and the use of antimicrobial agents to immunize the body against infectious and parasitic diseases (Mehta et al., 2014). Nature also plays its role in fighting against disease-causing microbes (Ibrahim et al., 2011; Bor et al., 2016). For example, many plants such as *Nigella sativa*, *Capsicum*, *Musa paradisiaca* L., and *Citrus limetta* have attracted tremendous public interest due to their efficient antimicrobial agents (Dutta et al., 2020b; El-Naggar et al., 2020; Ajijolakewu et al., 2021; Vanlalveni et al., 2021; Vijayakumar et al., 2021). Furthermore, whether in underdeveloped, developing, or developed regions of the world, these natural goods are widely accessible. Additionally, these items are cost-effective in their use because they don't need to be prepared using a time-consuming procedure in order to fight infectious bacteria (Ncube et al., 2008).

Nigella sativa, *Capsicum*, peels of *Musa paradisiaca* L., and *Citrus limetta* contain effective antimicrobial agents against various infectious microbes such as the following: pathogenic bacteria (gram-negative and gram-positive), including *S. aureus*, *Candida albicans*, *E. coli*, Gram-positive cocci, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton interdigitale*, four species of *Trichophyton rubrum*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Listeria monocytogene*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Pseudo. aeruginosa*, *Salmonella enteritidis*, *Sal. Typhimurium*, and *Shigella flexneri* (Hanafy and Hatem, 1991; Hosseinzadeh et al., 2007; Hannan et al., 2008; Halawani, 2009; Chaieb et al., 2011; Alshareef, 2019). The microbes listed above are known to cause the following diseases: pneumonia, bloodstream infections, wound or surgical site infections, methicillin-resistant *Staphylococcus aureus* (MRSA), urinary tract infection, "traveler's diarrhea," immunologic infections, diarrhea (sometimes bloody), fever, stomach cramps (Awaisheh and Ibrahim, 2009; Awaisheh et al., 2013; Blinov et al., 2022) and various others as reported in earlier conducted studies (Edelson and Unanue, 2000; Otto, 2009; Doernberg et al., 2017; Liu and Ji, 2018). These diseases can be prevented by using *Nigella sativa*, *Capsicum*, peels of *Musa paradisiaca* L., and *Citrus limetta* as antimicrobial agents. We have chosen to focus on these particular natural resources due to their global availability.

Worldwide, *Capsicum* grows on 1.93 million hectares of surface area. China is the world's largest producer of *Capsicum* at ∼16 million tons annually. Mexico is the second largest producer at ∼2.3 million tons, followed by Turkey and Indonesia with 2.2 and 1.8 million, respectively (Penella and Calatayud, 2018). *Nigella sativa* is produced mainly by India on 9,000 hectares. It is also produced in Pakistan, Sri Lanka, Nepal, Bangladesh, Egypt, and Iraq (Huchchannanavar et al., 2019). The annual global production of *Musa paradisiaca* L. is ∼41.265 million tons per year with its primary producers being Brazil, China, Ecuador, the Philippines, and India (Preedy and

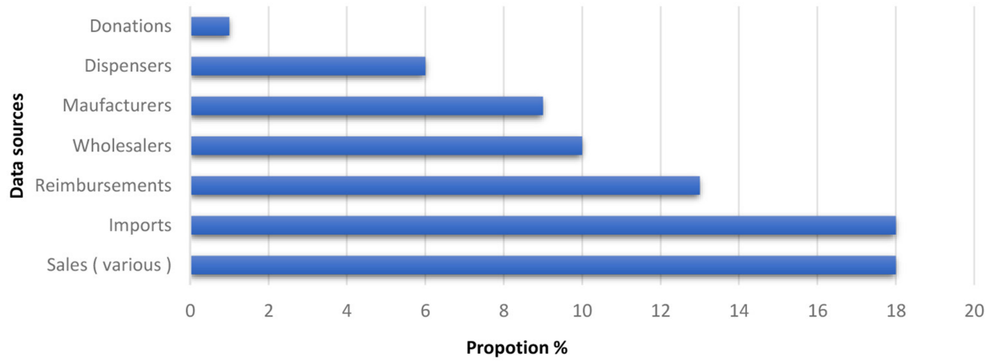

FIGURE 1
National estimates of antimicrobial consumption in 65 countries and areas (Organization, 2018).

Watson, 2019). Brazil is the largest *Citrus limetta* producing country globally with 15,912 tons followed by China with 5,450, the United States with 9,237 and the European Union (EU) with 5,999 thousand metric tons (Spreen, 2010). Annually, the global production of *Musa paradisiaca* L. is around 41.3 million tons and a large fraction of it is wasted as the peel. Notably, just in Brazil ∼780 million tons of *Musa paradisiaca* L. -waste is produced annually (Martínez-Ruano et al., 2018). According to Prabhakar and Singh (2019), ∼57% of processed *Citrus limetta* contributes to peel waste Usage of the endocarp of *Musa paradisiaca* L. and *Citrus limetta* creates solid waste problems due to the production of their peels, whereas *Nigella sativa* and *Capsicum* do not create such a problem. In this review, we address the handling of the huge solid waste problem produced by *Musa paradisiaca* L. and *Citrus limetta* by utilizing their peels as an antimicrobial agent against infectious diseases.

At present globally, a gradual increase in the wide spread of infectious diseases is quite evident. Therefore, it is need of time to adopt suitable preventive measures, such as regular use of antimicrobial agents. Therefore, it is proposed in this review paper that *Capsicum, Nigella sativa, Musa paradisiaca* L., and *Citrus limetta* on account of their abundant availability and antimicrobial constituents should be regarded as potential raw materials for the economical commercial production of various antimicrobial agents (Parthiban et al., 2011; Somda et al., 2011; Chitranshi et al., 2020). These natural materials are non-toxic, produce only organic food waste, and are easily available and completely safe for producing antimicrobial agents. Furthermore, the current study also discusses the antimicrobial compounds present in *Capsicum, Nigella sativa,* peels of *Musa paradisiaca* L., and *Citrus limetta* along with the methods employed for their extraction. Moreover, we also explain the overall antimicrobial mechanism of antimicrobial agents present in *Capsicum, Nigella sativa, Musa paradisiaca* L., and *Citrus limetta.* A schematic illustration of the content in this review is presented in Figure 2.

Biological properties of plant

The history of plants and/or plant components' utilization to flavor and conserve food, and to prevent oxidation and treat health problems is dated back to thousands of years ago. Most interestingly, the knowledge of their biological properties has been transmitted over the centuries among human societies. Considering this fact, it has been stated that the phytochemicals are the valuable source of bioactive substances with significant antimicrobial and antioxidant properties (Silva and Fernandes, 2010; Radha and Laxmipriya, 2015).

Anti-microbial agents present in plants

It has long been believed that plants have therapeutic properties. Since prehistory, people have used hundreds of thousands of local plants in infusions and poultices to treat various ailments. The desire to find novel antimicrobials has been prompted by the rise of microbial strains that are resistant to antibiotics and the recurrence of newer, deadlier illnesses. To find possible pharmacological leads, the whole natural resource base is being quickly evaluated (Ref: Antimicrobial Agents from Plants Reshma Reghu, Pramod Sahadevan & Shiburaj Sugathan + Plant Products as Antimicrobial Agents Marjorie Murphy Cowan*). Many plants and/or plant components, including *Nigella sativa, Capsicum,* peels of *Musa Paradisiaca* L., and *Citrus limetta* are ascribed to the presence of anti-microbial agents, which will be discussed herein.

Antimicrobial agent in *Nigella sativa*

It contains more than 100 valuable elements. According to science *N. sativa* contains 20–85% of protein, 38.20% of fat, 7–94% of fiber, and 31.94% of total carbohydrates that have antimicrobial effects. Due to the presence of thymoquinone and

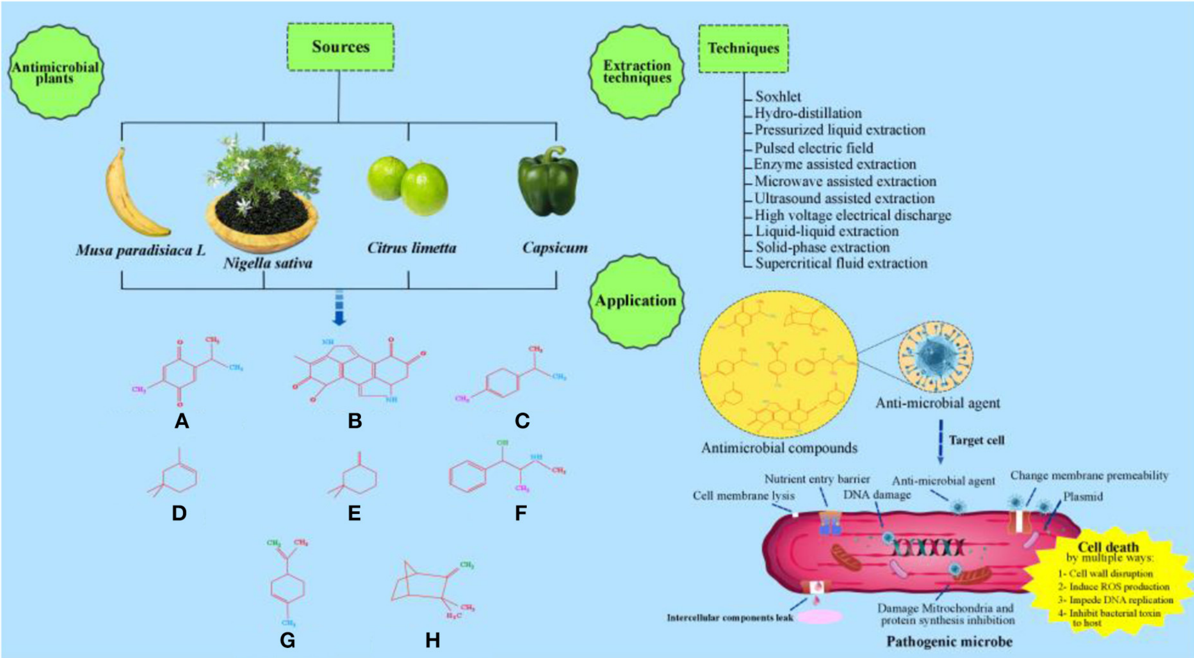

FIGURE 2
Schematic illustration of antimicrobial effects of *Capsicum, Nigella sativa, Musa paradisiaca L and Citrus limetta.* **(A)** Thymoquinone. **(B)** Melanin. **(C)** P-cymene. **(D)** a-Pinene. **(E)** b-Pinene. **(F)** Alkaloid. **(G)** Limonene. **(H)** Camphene.

thymohydroquinone in *N. sativa* it can fight against infectious disease-causing microbes (Bakal et al., 2017). The amount of the most significant bioactive component, thymoquinone, found in the volatile oil obtained from the seeds of *N. sativa* using various extraction techniques can range from 1.06 to 8.8 mg/g of oil, respectively (Yimer et al., 2019).

Antimicrobial agent in *Capsicum*

Capsaicin, also known as 8-methyl-N-vanillyl-6-nonenamide, is a compound found in *Capsicum* plants (chili peppers), which are used for food and medicine. Due to its antibacterial and anti-virulence properties, as well as its multiple pharmacological and physiological benefits (pain alleviation, cancer prevention, and advantageous cardiovascular, and gastrointestinal benefits; Marini et al., 2015), capsaicin has recently attracted a lot of attention. The capsaicinoids (2.5 mg/g; Othman et al., 2011) found in capsicum, which include more than 20 alkaloids (Bakht et al., 2020), are of major medical significance worldwide. Since capsicum has potent antibacterial effects, it is beneficial to utilize it in common food items.

Antimicrobial agent in *Musa paradisiaca* L.

Ethanol (0.5 g/100 g; Gorgus et al., 2016), which has a microbe-killing effect, is present in *Musa paradisiaca* L. at a concentration of 0.5 g/100 g. Secondary metabolites such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids (Imam and Akter, 2011) have been found to be present in banana peel. Ighodaro (2012) evaluated the antibacterial effectiveness of banana peel extract against human pathogenic microorganisms and exhibited good inhibitory activity against *S. aureus*, *Escherichia coli*, and *Proteus mirabilis*. Although banana peel is regarded as waste, peel extracts can be utilized to create antibiotics (Saleem and Saeed, 2020).

Antimicrobial agent in *Citrus limetta* peel

Peel from *Citrus limetta* can be utilized to make solid waste management more efficient as well as to create long-lasting antibacterial products. Citrus peel has been discovered to have thousands more phenolic compounds than citrus pulp (Dutta et al., 2020a), and these phenolic compounds (444.55–502.54 mg/L; Buyukkurt et al., 2019) have antimicrobial properties. In a 2009 study, Kekuda et al. investigated the antifungal efficacy of the peels of three distinct citrus fruits, *C. limetta*, *C. sinensis*, and *C. limon*, against *Aspergillus* species, and found that *C. limetta* was best at preventing the growth of the fungus that was tested (Shabnam et al., 2013).

Plants and/or plant components derived antimicrobial compounds

Thymoquinone, melanin, P-cymene, a-pinene, b-pinene, alkaloid, limonene, and camphene are significantly present in *Nigella sativa, Capsicum, Musa Paradisiaca* L., and *Citrus limetta.* Moreover, these plant-derived chemicals are known to act as antimicrobial agents with numerous applications in the medical and food industries, which will be discussed in the subsequent section.

Thymoquinone

Thymoquinone is a compound that has remarkable anti-sepsis activity at a specific dose. Thymoquinone is the main component present in *Nigella sativa* that is found to be effective against the Avian influenza virus and coronavirus (Ulasli et al., 2014). Another study found that *Nigella sativa* consumption makes it difficult for the coronavirus to survive and replicate in the body (Ahmad et al., 2020). Thymoquinone is reported as an inhibitor for the treatment of coronavirus infections by blocking the entry of virus into the cell (Xu et al., 2021). Studies reported that thymoquinone can serve as an anticancer, anti-inflammatory, antioxidant and as an analgesic compound. Activator of transcription (JAK-STAT), janus kinase/signal transduction, mitogen-activated protein kinase (MAPK), and nuclear factor kappa beta (NF-κβ) signaling pathways can be inhibited by thymoquinone. Thymoquinone causes disruption in reactive oxygen species (ROS), pro-inflammatory cytokines, lipooxygenase (LOX) enzymes, cyclooxygenase (COX), myeloperoxidase, and elastase (Ali A. et al., 2021).

Melanin

Melanin is an efficient green agent that possesses anti-inflammatory, antioxidant, and antimicrobial activity with vast application in the biomedical field (Silvestri et al., 2017; Avossa et al., 2021). Antioxidants protect human bodies from free radicals, which may otherwise result in causing various diseases such as cancer and coronary heart disease (Hamid et al., 2010). Additionally, it is promising as an antibacterial agent against both gram-positive and gram-negative pathogens (Liu et al., 2014).

P-cymene

P-cymene is a natural compound found in many plants. It is used as an important agent in many drugs. It is widely present in more than 200 types of foods, including grape fruit, orange juice, mandarin, raspberries, carrots, butter, oregano, nutmeg, and almost every spice. P-cymene can serve as an analgesic, anti-inflammatory, antioxidant, and anti-tumor agent (Nickavar et al., 2014). P-cymene has anti-nociceptive, anticancer, antidiabetic, antiviral, antiparasitic, anti-inflammatory, antifungal, antioxidant, and antibacterial effects (Balahbib et al., 2021).

Pinene

Pinene is the natural compound found in citrus fruits. Pinene has various potential benefits such as it has anti-inflammatory, antimicrobial, antioxidant, and neuroprotective effects (Di Rauso Simeone et al., 2020). Pinene has anticoagulant, anti-inflammatory, anti-leishmania, antimalarial, antimicrobial, antioxidant, antitumor, analgesic, and antibiotic resistance modulation effects (Park et al., 2021). Pinene has the wound healing process by generating scars with effective tensile strength, accelerating wound closure, acting as an adhesive of primary intention, and contributing to collagen deposition (Salas-Oropeza et al., 2021).

Alkaloid

Alkaloid is a natural compound found in many plants. The presence of alkaloids in plants protects them from the destruction of various insects (Zhang et al., 2005). Indole alkaloids and isoquinoline alkaloids are the primary classes of substances having antibacterial action among the numerous alkaloids, including pyridine alkaloids, indole alkaloids, steroidal alkaloids, and other alkaloids. Alkaloids have antiviral, antibacterial, antifungal, cytotoxic, antioxidant, antifouling activities and anti-infective potential (Barghout et al., 2020; Diaz et al., 2020; Youssef et al., 2021).

Limonene

Limonene is a chemical found mostly in citrus fruits (Rodríguez et al., 2011). Limonene has several health benefits and belongs to the group of terpenes. These chemicals have strong aromas which protect plants from predators. Limonene also has anti-inflammatory and antioxidant effects (Miguel, 2010). Limonene can be used as a greener and safer alternative to market-available antimicrobial agents (Ibáñez et al., 2020).

Camphene

Camphene is a monoterpene found in many plants (Russo and Marcu, 2017). Terpenes are those chemicals that have strong aromas which protect plants from predators (Miguel, 2010). Camphene can fight against various deadly pathogens, such as *Enterococcus spp*. and *S. aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and have an antifungal effect against *Trichophyton mentagrophytes* and several species of *Paracoccidioides spp*., Camphene have activity against *Mycobacterium tuberculosis* and vancomycin resistance

Enterococcus spp. Isolates (de Freitas et al., 2020). Figure 3 shows the chemical structures of thymoquinone, melanin, P-cymene, a-pinene, b-pinene, alkaloid, limonene, and camphene.

Table 1 shows the major antimicrobial compounds present in *Nigella sativa* and *Capsicum* along with the adopted methodology and important findings reported in previous research studies. Whereas, Table 2 shows the major antimicrobial compounds present in *Musa paradisiaca* L. and *Citrus limetta* along with the adopted methodology and important findings reported in previous research studies.

Extraction of antimicrobial compounds

The initial three basic processes in the extraction of antimicrobial compounds reported in the literature are (1) pretreatment, (2) extraction, and (3) purification.

Pretreatment

The first step in the extraction of antimicrobial agents is the preparation of materials employing methods such as washing, grinding, dewatering, heating and microfiltration. Pre-treatment consists of three additional steps: physical treatment, chemical treatment, and biological treatment.

Physical treatment

Physical treatment involves pre-washing, drying, grinding to get a homogeneous sample, heating, steaming, freeze-drying, and often boosting the kinetics of analytical extraction as well as increasing the contact of the sample surface with the solvent system. When making the extract from plant samples, proper precautions must be taken to ensure that any potential active ingredients are not lost, altered, or destroyed (Fabricant and Farnsworth, 2001; Cos et al., 2006; Sasidharan et al., 2011).

Chemical treatment

Chemical treatment involves the use of salts, acids, bases, and solvents. The chemical extraction method relies on the kinds of functional groups each component in the mixture possesses. Chemical processes can separate or purify materials when the right reagents are used. There are several chemical tests that can be used to identify the presence of alkaloids, flavonoids, tannins, saponins, flavones, sterols, and terpenes, which have been briefly discussed in the later part.

Identification of alkaloids

The extracts are individually filtered, dissolved in diluted hydrochloric acid, and analyzed for the presence of alkaloids. Typically, Mayers and Wagner's tests are used to identify alkaloids. In Mayer's test, the appearance of a yellow color on using Mayer reagent indicates the presence of alkaloids. Whereas, in Wagner's test a brown-reddish brown is developed upon using Wagner's reagent that indicates the presence of alkaloids (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of flavonoids

For the identification of flavonoids lead acetate and sulphuric acid test are usually performed. In Lead acetate test; the extracts are mixed with a few drops of a lead acetate solution. The presence of flavonoids is indicated by a precipitate that is yellow in color. In the sulfuric acid test; after a couple of drops of sulfuric acid are added to the extracts, an orange color appears, indicating the presence of flavonoids (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of steroids

Likewise, on adding a few drops of acetic anhydride to the extracts causes samples to turn violet then blue and lastly to green, which is a sign of the presence of steroids (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of terpenoids

The identification of terpenoids is usually done by Salkowski's test. This test is conducted by adding 3 mL of concentrated sulfuric acid to an extract of 5 mg of the targeted plant component. Eventually, the presence of terpenoids is indicated by the appearance of reddish-brown color (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of anthraquinones

For the identification of anthraquinones, Bontrager's test is usually performed. The Bontrager's test involves boiling 5 mg of the extract in a water bath for a short period of time with 10% HCl. After filtering, it is allowed to cool followed by the addition of $CHCl_3$ in an equal amount to the filtrate. The liquid is then heated before a few drops of 10% NH_3 are added. Anthraquinones are present when a pink color forms, indicating their presence (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of phenols

For the identification of phenols, ferric chloride test and lead acetate test are usually performed. In the Ferric chloride test; 10 mL extract is mixed with a few drops of ferric chloride. The presence of phenol is indicated by a bluish-black color. In the Lead acetate test; 10 mg of extract is added with a few drops of lead acetate solution. The presence of phenol is indicated

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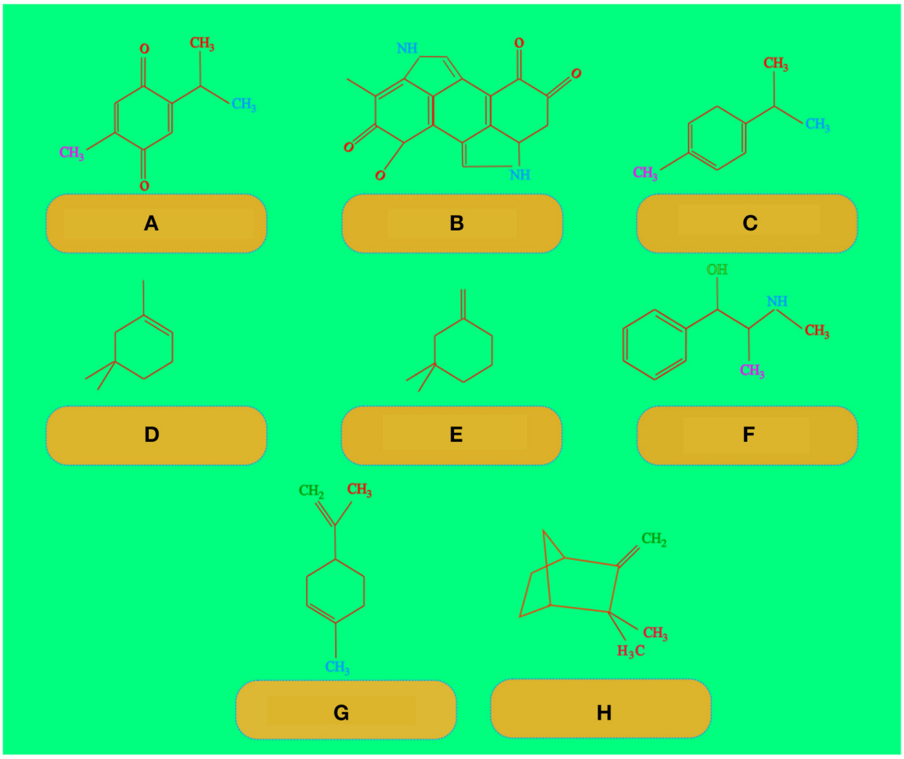

FIGURE 3
Chemical structures of **(A)** thymoquinone, **(B)** melanin, **(C)** P-cymene, **(D)** a-pinene, **(E)** b-pinene, **(F)** alkaloid, **(G)** limonene, and **(H)** camphene.

by a yellow color (Trease and Evans, 1989; Wallis, 1989; Al-Owaisi et al., 2014; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Biological treatment

Biological treatment uses bacteria and fungi to extract antimicrobial agents. The antibacterial activity of an extract or a pure chemical can be assessed or screened using a number of biological laboratory techniques. Disk-diffusion and broth or agar dilution procedures are the most well-known and fundamental techniques (Magaldi et al., 2004; Pfaller et al., 2004; Roden et al., 2005; Das et al., 2010; Bensah and Mensah, 2013; Kokollari et al., 2015; Fierascu et al., 2019).

Disk-diffusion method

The underlying idea behind this procedure is that an antibiotic concentration gradient is created when an antibiotic-impregnated disc is placed on agar that has already been inoculated with the test bacterium. The antibiotic is present in high concentrations near the disk's edge and gradually decreases as the space between them expands until the or3ganism is no longer inhibited by the antibiotic, at which time it is free to proliferate. If the antibiotic suppresses bacterial growth, a clear zone or ring forms around the antibiotic disc incubated overnight (Digrak et al., 1999; Parekh et al., 2005; Klančnik et al., 2010; Novaković and Janković, 2011).

Broth and agar dilution

Agar dilution implies adding various quantities of the antimicrobial drug to a nutrient-rich agar medium before applying a set number of cells to the agar plate's surface. In broth dilution, Bacteria are introduced into a liquid growth medium while being exposed to various concentrations of an antimicrobial agent, which is frequently assessed in 96-well microtiter plate format. After 16–20 h of incubation, growth is evaluated and the MIC value is recorded. This 3-day regimen is only applicable to aerobic bacteria (Griffin et al., 2000; Wiegand et al., 2008; Wu C. et al., 2015; Albano et al., 2020).

Extraction techniques

The second step is the extraction of required compounds from the material. The best extraction process must involve the thorough extraction of all the necessary metabolites or chemicals. If it's going to be done repeatedly, it needs to be quick, easy, and repeatable. The choice of an appropriate extraction technique mostly depends on the work that has to be done

TABLE 1 *Nigella sativa* and *Capsicum* as an antimicrobial agent.

Name of material	Antimicrobial agent	Adopted methodology	Findings	References
Nigella sativa	Thymoquinone and melanin	Paper disc diffusion method	A clear antibacterial effect on the growth of *Staphylococcus* was obtained	Bakathir and Abbas, 2011; Alshareef, 2019
	Fixed oil and thymoquinone of *N. Sativa*	-	Effective against many microbes including *Escherichia coli*, *Shigella niger*, *Staphylococcus Albus*, *Salmonella typhi*, and *Vibrio cholera*. Most effective against *Aspergillus species*	Ali and Blunden, 2003
	Thymoquinone, p-cymene, p-tert-butylcatechol, and pinene	Disc diffusion method	Thymoquinone is the main component that causes antimicrobial effects	Roy et al., 2006
	Essential oil of *N. sativa*	Plate diffusion method	Most effective against *Bacillus subtilis*	El-Kamali et al., 1998
	Crude alkaloid and water extract of the seed	-	Gram-negative was most effective than Gram-positive	Morsi, 2000
Capsicum	Low pH of fruit	Disk diffusion method and Well diffusion method	Extracts of *Capsicum* fruits are suitable for antibacterial activities	Careaga et al., 2003; Tano et al., 2008; Koffi-Nevry et al., 2012
	Isopropanol extracts	Growth inhibition test	Cinnamic and M-coumaric acids from Chili extracts are effective against bacterial species.	Dorantes et al., 2000
	Capsaicin-induced alterations in the pH	-	Prevent microbial infections	Tellez et al., 1993
	Presence of Capsaicin	Inhibit CT production	Effective against *V. cholera*	Omolo et al., 2014
	Presence of Capsaicin and Capsaicinoids	Disk diffusion method	Capsaicinoids are main component that causes antibacterial effect.	Das et al., 2018

and on the target metabolites to be known. There are various extraction processes like; Conventional techniques include solvent extract, soxhlet extraction, vortexing, maceration, centrifugation, hydro-distillation, cold pressing, and pressing and solid-liquid dynamic extraction. Non-conventional techniques include; pressurized liquid extraction, microwave-assisted method, ultrasound-assisted method, pulsed electric field, enzyme-assisted extraction, and supercritical fluid extraction. Conventional techniques are more time-consuming and less environmental friendly while non-conventional are fast and environment friendly techniques (Koubala et al., 2008; Azmir et al., 2013a,b; Deng et al., 2015).

Extraction technologies for extracting antimicrobial agents from *Nigella sativa, Capsicum*, peels of *Musa paradisiaca* L., and *Citrus limetta*

This review presents an analysis of several extraction techniques to extract antimicrobial agents from different plant sources, especially *Nigella sativa*, *Capsicum*, peels of *Musa paradisiaca*, and *Citrus limetta*.

Soxhlet extraction

This extraction technique involves putting solid material inside the thimble present in the extractor. The solvent condenses and fills the thimble as the vapor rises. This process of extraction is repeated again and again until the desired quantity is achieved. This technique was firstly used for the determination of fat in milk (Voon et al., 2012; Parashar et al., 2014; Chen and Urban, 2015). Five types of soxhlet techniques are mainly used for solvent extraction i.e., conventional soxhlet, high-pressure soxhlet extraction, ultrasound-assisted soxhlet extraction, automated soxhlet extraction, and microwave-assisted soxhlet extraction (Redfern et al., 2014; Wu C. et al., 2015; Wu G. et al., 2015). Conventional soxhlet extraction is a continuous process with slow processing time and an environmental unfriendly technique (Azmir et al., 2013b; Lopresto et al., 2014). High-pressure soxhlet extraction is used to fractionate polyethylene. The automated soxhlet technique saves

TABLE 2 *Musa paradisiaca* L. and *Citrus limetta* as an antimicrobial agent.

Name of material	Antimicrobial agent	Adopted methodology	Findings	References
Musa paradisiaca L.	Alcoholic extracts of *Musa paradisiaca* banana fruit peel	Agar diffusion method	Effective against *Staphylococcus* and *Pseudomonas*	Ahmad and Beg, 2001; Karuppiah and Mustaffa, 2013
	Presence of Phenolic and Flavonoid compounds	Minimum Inhibition Concentration (MIC) and paper disc diffusion method	ß-sitosterol, 12-hydroxy strearic, malic and succinic acid from banana green peel shows antibacterial activities	Fidrianny et al., 2014
	Presence of fatty acids	Fermentation	A suitable environmental condition should be provided for high ethanol production	Brooks, 2008
	Methanol extract of *M. paradisiaca* peels	Zone of inhibition test (ZIT), minimum bacterial concentration (MBC) and minimum inhibitory concentration (MIC)	Methanol extracts of Banana peels are more effective against bacteria than its ethanol extracts.	Okorondu et al., 2010
Citrus limetta	Aqueous and organic extracts of *C. limetta* peels	Solvent extraction procedure	Methanolic extract of *Citrus limetta* have antibacterial activity mostly against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*	Shakya et al., 2019; Dutta et al., 2020a
	Ethyl acetate and butanol fractions of *C. limetta* peels extract	Agar diffusion method	Ethyl acetate stops the growth of bacteria and butanol fractions stops the quorum sensing control pigment production	Khan et al., 2017
	Limonene (95.98%) Camphene (1.79%) and Terpenes	Hydro-distillation, Disc diffusion method	Have strong antibacterial activity against food-borne pathogens	Javed et al., 2013
	Peel extracts	Well diffusion assay	Pomegranate peel extract showed maximum antibacterial activity among Orange, Banana, Sweet lime, Apple, Papaya, and Mango	Prakash et al., 2013
	Acetone extracts	Disc diffusion method	Effective against *Staphylococcus aureus Bacillus subtilis*, and *Bacillus* cereus	Al-Snafi, 2016

time, sample consumption and is used for agricultural, food and industrial samples. Ultrasound-assisted soxhlet extraction is mainly used to extract fat from sunflowers, grapes and soya beans. However, this technique does not affect the quality of extracted oil due to the adoption of mild conditions in the process (De Castro and Priego-Capote, 2010; Parashar et al., 2014).

The soxhlet technique was used to extract 6.77 ± 1.2, 3.66 ± 1.43, and 4.81 ± 1.8% w/w of thymoquinone from *Nigella sativa* (Kausar et al., 2017). Capsaicin was extracted from *Capsicum* by using this technique (Chuichulcherm et al., 2013). *Citrus limetta* which is a major phyto compound was extracted from *Capsicum* by using this technique (Baranitharan et al., 2020).

Hydro distillation

Hydro distillation is a conventional method that is used for extracting bioactive compounds from different plant species. This process involves packing plant materials in a still chamber, adding an appropriate amount of water, and then heating it to a boil. Alternative methods include injecting direct steam straight into the plant sample. By using indirect cooling with water, the water and oil vapor combination is condensed. Oil and bioactive substances are automatically separated from the water in a separator when the condensed mixture flows from the condenser. Its usage is constrained by restrictions on high-temperature applications for heat-sensitive phenolic compounds, although it has several benefits such as the lack of organic solvents in the process, the requirement for no dehydration of the plant materials, and faster extraction times (Vankar, 2004; Sa-Nguanpuag et al., 2011; Erkan et al., 2012; Chenni et al., 2016; Elyemni et al., 2019).

Through this technique, thymoquinone and capsaicin were extracted from *Nigella sativa* (El Khoury et al., 2019) and *Capsicum,* respectively (Jiang et al., 2013). Hydro distillation is the most common method for obtaining thymoquinone from *Nigella sativa* (Liu et al., 2011; Oskouei et al., 2018).

Liquid–liquid extraction

In liquid-liquid extraction separation of required material from liquid solution is made through contact with another insoluble liquid. The required material is separated based on its solubility in different liquids (Chen et al., 2017; El Blidi et al., 2019; Goyal and Singh, 2022).

It is best suited for liquid samples. The technology is simple and can be used at ambient temperature. However, the technology is time-consuming, requires expensive chemicals, and takes a lot of labor (Espinosa-Alonso et al., 2006).

Liquid-liquid extraction is an energy e?cient technique (Blahušiak et al., 2018) and can be used in biorefineries for lipid extraction from microalgae (Du et al., 2017) and for fractionation of pyrolytic bio-oils (Cesari et al., 2019). Earlier research study also reports lignin recovery *via* liquid extraction (Stiefel et al., 2017). Moreover, the fractionation of citrus essential oils (separating the terpenic hydrocarbons and oxygenated compounds) is achieved through liquid-liquid extraction (Gonçalves et al., 2016).

Solid-phase extraction

The solid-phase extraction technique is the simplest technique for extracting and pre-concentrating trace levels of contaminants from samples. The extraction efficiency of solid-phase extraction is enhanced by using nanomaterials as sorbents which provide more pollutants capturing sites (Jacobsen et al., 2004; Silva et al., 2016; Azzouz et al., 2018).

The only difference between solid-phase extraction and liquid-liquid extraction is the medium used for separation; in solid-phase extraction, one phase is a liquid phase and the other is a solid phase. The solid surface consists of adsorbent material that absorbs specific required material through the solution. The separation rate of solid-phase extraction technique is faster than liquid-liquid extraction technique. It is a simple technique. It can be employed for polar compounds. It is not suitable for volatile samples (Abd-Talib et al., 2014). It is an expensive technique than the liquid-liquid extraction technique (Hernanz et al., 2008). Different methods were used to extract thymoquinone from *Nigella sativa* including Solid-phase extraction (Kausar et al., 2017).

Supercritical fluid extraction

Suppose the temperature and pressure of a specific liquid and gas are above the critical point. In that case, the supercritical fluid extraction technique should be used for extracting certain required materials from samples like essential oils. Certain parameters such as temperature, pressure, the flow rate of fluids, and the sample size significantly affect extraction yield (Santoyo et al., 2006; Ma et al., 2018; Yousefi et al., 2019; Zizovic, 2020; Santos et al., 2021).

The supercritical fluid extraction technique saves time, it is sustainable and suitable for volatile samples. However, it is not suitable for drugs and polar substances. It is also a costly technique (Mendiola et al., 2007; Abbas et al., 2008).

From mandarin, orange, lemon, grapefruit peel and sour orange peel phenolic compounds were extracted by using supercritical fluid extraction (Omar et al., 2013; Trabelsi et al., 2016). Super critical fluid extraction is used for obtaining thymoquinone from *N. sativa* (Liu et al., 2011; Oskouei et al., 2018).

Pressurized liquid extraction

Pressurized liquid extraction is mainly used for extracting phenolic compounds from plants. Liquid solvents are used in it with specific temperatures and pressure. It is suitable for solid samples and polar compounds. However, pressurized liquid extraction is time-consuming technology that requires less solvent. Also due to the 10 g maximum sample weight limit, it is not appropriate for situations where very low-level analyte detection is required (Jacobsen et al., 2004; Juan et al., 2010; Mustafa and Turner, 2011; Alañón et al., 2015; Alvarez-Rivera et al., 2020). It is mainly used to recover bioactive compounds from grapes (Pereira et al., 2019). Extraction of phenolic compounds (alkaloids) from *Citrus limetta* through pressurized liquid extraction was carried out by Olabinjo et al. (2020).

Pulsed electric field

The pulsed electric field is an efficient non-thermal technique that involves the application of high-voltage pulses. These pulses cause rupturing of plant materials thus extraction of required components occurred from plant cells. Though it is a sustainable technology, it takes a long time to process. This technology requires process parameters like energy inputs, treatment temperature and field strength to be maintained (Liang et al., 2002; Wu et al., 2005; Nguyen and Mittal, 2007; Puértolas et al., 2012; Garner, 2019). According to a study, *Citrus limetta* peels were subjected to pulsed electric fields in order to extract phenolic components (Luengo et al., 2013). In another study, phenolic compounds were extracted from orange, grapefruit, and lemon peel through a pulse electric field (El Kantar et al., 2018). Much more components were extracted from the *N. sativa*'s oil when PEF was applied as a pretreatment. The major constituent was thymoquinone and cymene, whereas D-Limonene and β-Pinene were also present in small quantities (El-Dakhakhny et al., 2000; Bakhshabadi et al., 2018).

Enzyme-assisted extraction

Specific enzymes are used in enzyme-assisted extraction to target the cell walls of plant materials, destroying them and liberating antimicrobial compounds. The effectiveness of the procedure as a whole can be improved by combining this strategy with a number of other techniques (Nadar et al., 2018; Bilal et al., 2019; Habeebullah et al., 2020). For instance, Naghshineh et al. (2013) found enzymatic extraction with high pressure treatment led to a higher level of pectin extraction form lime peel powder (Naghshineh et al., 2013). This technology has high extraction rates and is sustainable. However, it has a high enzyme cost and is not appropriate for industrial use. Its operations must be handled with caution (Puri et al., 2012; Singh et al., 2016). Camphene is the predominant essential oil of *Aristolochia manshurientsis Kom* extracted from enzyme-assisted extraction followed by hydrodistillation (Zhao et al., 2018).

Microwave-assisted extraction

Organic compounds can be extracted from plant material with more ease and effectiveness using microwave technology than using traditional methods. It is a technology that saves time. It is a very efficient technology with a high yield. This technology is sustainable because it requires less heat (Huie, 2002; Kaufmann and Christen, 2002; Wang and Weller, 2006; Chen et al., 2007; Cravotto et al., 2008; Sticher, 2008; Zhang et al., 2009). It involves a simple and economical process as compared to supercritical fluid extraction. It has a difficult operation as compared to ultrasonic-assisted extraction (Hayat et al., 2009). Extraction of Capsaicin through *Capsicum* was done through microwave-assisted extraction by Chuichulcherm et al. (2013). A study reported that phenolic compounds (alkaloids) were extracted from *Citrus limetta* through microwave-assisted extraction (Arafat et al., 2020). In another study, phenolic compounds were extracted from orange peel through microwave-assisted extraction (Nayak et al., 2015). Microwave-assisted extraction is used for obtaining thymoquinone from *N. sativa* (Liu et al., 2011; Oskouei et al., 2018). Various alkaloids were extracted from plants using microwave-assisted extraction (Bergs et al., 2013; Ngo et al., 2017; Aqil et al., 2020).

Ultrasound-assisted extraction

Ultrasound-assisted extraction is a technique that involves throwing sound energy to plants and agitating it thus extracting the required materials through a plant cell. In this technology, low energy and chemicals are required. High yield can be obtained from it, and it is a time-saving technology. Proper optimization in ultrasound frequency, the nominal power of the device, input power, propagation of cycle, and system geometry is required for maximum yield (Azmir et al., 2013a; Barba et al., 2015; Sneha et al., 2022). Studies report the extraction of Capsaicin from *Capsicum,* phenolic compounds (alkaloids) from orange peel and citrus fruit peel through ultrasound-assisted extraction (Chuichulcherm et al., 2013; Omar et al., 2013; Boukroufa et al., 2015; Nishad et al., 2019). Different methods were used to extract thymoquinone from *Nigella sativa* including Ultrasound-assisted extraction (Kausar et al., 2017).

High-voltage electrical discharge

A high-voltage electrical discharge is a non-thermal method that relies on ambient temperature and uses pulsed rapid discharge voltages. It is a technique that saves time and uses less energy. However, at the industrial and pilot level, this technology has not yet been utilized (Barba et al., 2015; Li et al., 2019; Rajha et al., 2019; Žuntar et al., 2019). Extraction of phenolic compounds (alkaloids) from grapefruit peel (El Kantar et al., 2019) and from the mandarin peel (Buniowska et al., 2015), through high voltage electric discharge was studied in respective studies.

Comparative analysis of different extraction technologies for extracting bioactive compounds

Selecting an extraction technique presents one of the biggest challenges in getting bioactive compounds from plants because many factors need to be taken into account, including the solvent's polarity, the chemical makeup of the bioactive compound, yield, selectivity, temperature, solubility, extraction time, and cost. Natural plant extracts can be obtained in a variety of ways, but the elements of the process profoundly affect the final product's quality. Among the extraction techniques, the supercritical extraction and/or the extraction utilizing pressurized fluids can be highlighted. In these procedures, a highly compressible fluid is used as a solvent in low- or mid-temperature conditions. The utilization of supercritical fluids is a possible technique for the extraction and fractionation of natural resources, particularly for food and pharmaceuticals. The key advantages of supercritical fluid extraction include high selectivity, ease of solvent removal, and use of mild temperatures (Correa et al., 2017). Using *Capsicum frutescens* L. (Chuichulcherm et al., 2013), investigated the extraction of capsaicinoids using microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), in comparison to the conventional soxhlet method. They concluded that the UAE approach was the best technique for extracting capsaicinoids from *C. frutescens*. Therefore, even though the amounts of capsaicinoids in MAE and UAE were 5.28 and 4.01 mg/g of *C. frutescens*, respectively, UAE needed the least amount of energy and was deemed the best approach. The low-quality oil produced by traditional solvent extraction methods necessitates intensive purifying procedures, which cause thermal deterioration and the loss of important components. There has been a shift toward more environmentally friendly extraction techniques, such supercritical fluid extraction, as a result of the use of organic solvents and the need to recover the solvent (SFE). SFE is evolving into a successful and widely utilized method for extracting valuable natural compounds from challenging materials (Ghahramanloo et al., 2017). Particularly, maceration, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) have been considered as popular extraction techniques; Zhang and his coworkers concluded that MAE was the most effective with a yield of 7.04 ± 0.14 mg/g chelerythrine and 17.10 ± 0.4 mg/g sanguinarine with 5 min of extraction time (Zhang et al., 2005). High hydrostatic pressure (HHP), ultrasonics and pulsed electric fields (PEF) were used for extracting bioactive compounds from grapes and the finding of this study was; the total phenolic content of samples was 50% higher than in the control samples (Corrales et al., 2008). Typically, phenolics have antibacterial and antioxidant properties. Due to their unique molecular structure, can offer superior platforms for making antimicrobial products (Siddiqui et al., 2022). High pressure (HP), pulsed electric fields (PEF), and low pasteurization (LPT) were used for extracting, pulsed electric field and low pasteurization; the former was the most effective treatment for the extraction of bioactive components from orange juice (Plaza et al., 2011). Conventional soxhlet extraction, MAE and ultrasound assisted extraction (UAE) were used for extracting bioactive compounds from agiorgitico red grape pomace. This study concluded that UAE was the most effective one (Drosou et al., 2015). There have been several research investigations on the extraction of bioactive substances using traditional and non-conventional (green) methods, including soxhlet extraction, microwave, supercritical fluid extraction, ultrasound extraction, etc. Each methodology has pros and cons. For instance, the greatest yield (26.52%) and total phenolic content (15,263.32 mg Eq gallic/100 g DW) from citrus reticulata blanco cv. sainampueng peel were obtained *via* the extraction of bioactive components under the ideal UAE conditions. UAE therefore demonstrated greater extraction efficiency in terms of yield under the same extraction circumstances, which was 1.77 times higher than MAE (Saini et al., 2019).

Purification

Purification of antimicrobial agents is generally the final step. There are various purification methods that can be used to purify antimicrobial agents. The choice of a specific purification method in any study depends on the degree of purification required for specific antimicrobial agents. In the case of polar compounds, purification is done mainly by filtration, ultrafiltration, paper chromatography, thin-layer chromatography, gas chromatography and high-performance liquid chromatography. Whereas, non-polar compound purification is mainly performed by; liquid-liquid extraction, reversed phase purification chromatography and through Hydrophobic interaction chromatography (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Tang et al., 2018; Martinenghi et al., 2020).

Filtration

Filtration is a technique that involves pouring a combination of solid and liquid onto a membrane or a filter paper, which permits the passage of liquid (the filtrate) and results in the collection of the solid, this process is used to separate solids from liquids. During filtration, the size of the materials that can be removed relies on the filter's pore size. With just one filter, this technique can filter out a variety of undesirable particles of various sizes and is comparatively inexpensive (McGaw et al., 2002; Jin et al., 2007; Mushore and Matuvhunye, 2013; Sahle and Okbatinsae, 2017).

Ultrafiltration

Ultrafiltration (UF) is a type of membrane filtration, that forces a liquid against a semi-permeable membrane by hydrostatic pressure. Water and solutes <0.005 micro meter pass through the barrier, whereas suspended solids and solutes >0.1 micro meter are trapped. Compared to traditional methods, ultrafiltration technology can accomplish more work in 50% less space (Pampanin et al., 2012; Wang et al., 2013; Tang et al., 2018; Kellogg et al., 2019; Martinenghi et al., 2020).

Paper chromatography

In this method, separations are performed using a piece of paper that serves as both a support and a medium for separation. The filter paper is placed in the chromatographic chamber with the solvent, and the sample is put close to the bottom of the filter paper. By means of capillary action, the solvent flows while also transferring soluble molecules. Paper chromatography has the benefit that separations can be done easily on sheets of filter paper, which serves as both a substrate and a medium for separation. The high degree of reproducibility of Rf (retention factor) values can also be obtained on paper through this technique (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Muhamad et al., 2017; Tang et al., 2018; Martinenghi et al., 2020).

Thin-layer chromatography

Using this method, samples are separated based on their interactions with a thin layer of adsorbent that is adhered to the plate of low molecular weight substances. Compounds are separated using various adsorbents. The particular benefit of TLC over paper chromatography is its adaptability, speed, and sensitivity (Thumar et al., 2010; Galanakis, 2012; Tang et al., 2018; Martinenghi et al., 2020).

Gas chromatography

Utilizing this method, volatile substances are separated. Through its distribution in the gas phase, the chemical species' rate of kinetics is identified. While the liquid phase is still, the gas phase is moving. The chemical species' rate of migration is estimated based on how widely distributed it is in the gas phase. For instance, a species that disperses 100% of itself into the gas phase will migrate at the same rate as the gas that is flowing, whereas a species that disperses 100% of itself into the stationary phase will not move at all. In gas chromatography, a sample is vaporized and then injected into the chromatographic column's head. The flow of the inert, gaseous mobile phase carries the sample through the column. A liquid stationary phase that is adsorbed onto the surface of an inert solid is present in the column itself (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Tang et al., 2018; Sreedharan et al., 2019; Martinenghi et al., 2020).

High-performance liquid chromatography

HPLC is an analytical technique used for the separation and measurement of organic and inorganic solutes in any samples, particularly biological, pharmaceutical, food, environmental, industrial, etc. This method divides compounds by how they interact with the solvent of the mobile phase and the solid particles of a densely packed column. To help in identifying the chemicals, the Diode Array Detector examines the analytes' absorption spectra (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Tang et al., 2018; Martinenghi et al., 2020).

Reverse-phase chromatography

In reverse-phase chromatography (RPC), molecules are separated from one another through hydrophobic interactions between the ligands attached to the stationary phase and the solute molecules in the mobile phase of the liquid chromatography. Better solubility for polar analytes is provided by reverse-phase chromatography, which also uses non-toxic solvents, provides a means for removing impurities and mobile phase additives, and provides rapid sample recovery with minimal solvent evaporation (Nair and Kanfer, 2006; Thumar et al., 2010; Galanakis, 2012; Tang et al., 2018; Martinenghi et al., 2020).

Hydrophobic interaction chromatography

Based on their hydrophobicity, molecules are separated using hydrophobic interaction chromatography (HIC). Due to the use of less denaturing conditions and matrices, HIC is a good separation approach for removing proteins while preserving biological activity. This technique can improve the mass spectrometry's sensitivity of detection. When compared to reversed-phase liquid chromatography, the flow rate in this approach can be larger because the mobile phase's viscosity is low. Low viscosity mobile phases enable the use of columns that are twice as long as those required for reverse phase HPLC. The sample separates very quickly due to the eluent's viscosity, which is also correlated with a lower separation impedance. Compared to reverse phase HPLC, basic solutes can be loaded more readily (Thumar et al., 2010; Galanakis, 2012; Sagar et al., 2012; Tang et al., 2018; Martinenghi et al., 2020).

Antibiotic resistance mechanisms in disease causing bacteria

Microbial infections are on the rise globally, and the human population is at risk due to microbes because these microbes are the major cause of worldwide mortalities, as shown in Table 3. Basically, disease causing bacteria have resistance against various antibiotics due to myriad mechanisms. These mechanisms are: (1) Bacteria can have resistance through transformation, transduction, and conjugation phenomenon. (2) Through the processes of phosphorylation, adenylation, or acetylation bacteria deactivate the antibiotics. (3) Bacteria prevent the interaction of drugs and antibiotics. (4) They can cause efflux of the antibiotic from the cell (Munita and Arias, 2016; Reygaert, 2018; Abushaheen et al., 2020). Recently, resistance to antibiotics has been the biggest challenge which threatens human communities. Meanwhile, the occurrence of the evolution of resistance endangers the effectiveness of existing antibiotic drugs (Baym et al., 2016; Marston et al., 2016). Therefore, several strategies have been proposed to overcome the antimicrobial resistance. One of the recommended methods to achieve this goal is the usage of natural compounds that are derived from plants. These plant extracts seem to be ideally suited to overcome the emergence of antibiotic resistance in multidrug resistance bacteria (Ncube et al., 2008; Rossiter et al., 2017).

Anti-microbial properties of *Capsicum*, *Nigella sativa*, peels of *Musa paradisiaca* L., and *Citrus limetta*

Plants have a plethora of constituents that impose antimicrobial effects (Gyawali et al., 2011; Mickymaray et al., 2016; Dewapriya et al., 2018; Casciaro et al., 2019; Ali M. Y. et al., 2021). Two major groups of antibiotics extracted from plants are; (1) phytoanticipins and (2) phytoalexins. Phytoanticipins inhibit microbial actions, whereas Phytoalexins are generally antioxidants (Sukalingam et al., 2017, 2018). Moreover, various secondary plant metabolites are known to cause a significant antimicrobial effect which will be discussed in later section.

Antimicrobial mechanism of bioactive compounds

These secondary metabolites are grouped into three types; (1) phenolic compounds, (2) terpenes, and (3) alkaloids (Crozier et al., 2006; Mickymaray, 2019; Roaa, 2020). These antimicrobial extracts cause cell wall disruption and lysis, cell wall construction, inhibit biofilm formation, inhibit microbial DNA replication, inhibit energy synthesis, inhibit bacterial

TABLE 3 Infectious diseases caused by microbes.

Infectious disease	Microbe that causes the disease	Death rate	References
Methicillin-Resistant *Staphylococcus Aureus* (MRSA), Vancomycin-Resistant Enterococci (VRE), and *Clostridium difficile*	Gram-positive bacteria	76%	Doernberg et al., 2017; Sizar and Unakal, 2020
Pneumonia, bloodstream infections, wound or surgical site infections, and meningitis	Gram-negative bacteria	14%	Centers for Disease Control and Prevention, 2011; Sizar and Unakal, 2020
Candida can cause infections if it grows out of control or if it enters deep into the body (for example, the bloodstream or internal organs like the kidney, heart, or brain)	*Candida albicans*	19–24%.	Akpan and Morgan, 2002; Magill et al., 2014
Cholecystitis, Bacteremia, Cholangitis, Urinary tract infection (UTI), traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia.	*E. coli*	4.2%	Makvana and Krilov, 2015; Khalil et al., 2018
Dermatophytosis	*Trichophyton interdigitale*	4–8%	Kardjeva et al., 2006; Mügge et al., 2006; Zhang et al., 2019
Nosocomial infections	*Staphylococcus epidermidis*	20%	Otto, 2009; Turnidge et al., 2009
Food-borne bacterial illness cause to unborn babies, newborns and people with weakened immune systems	*Listeria monocytogene*	15–20%	Edelson and Unanue, 2000
Diarrheal and toxico-infections	*Bacillus cereus*	619 death from 1998 to 2015	Jessberger et al., 2020; McDowell et al., 2020
Superficial and ear infections in humans	*Vibrio alginolyticus*	35–50%	Reilly et al., 2011
Human infectious diseases including keratitis, burn infections, urinary tract infections (UTIs), sepsis, as well as acute and chronic infections of human airways.	*Pseudo. aeruginosa*	18–61%	Kang et al., 2003; Cao et al., 2017
Gastroenteritis and focal infections	*Salmonella typhimurium*	3 million each year	Coburn et al., 2007; Jung et al., 2020
Diarrhea (sometimes bloody), fever, and stomach cramps.	*Shigella flexneri*	13.2%	Khalil et al., 2018; Duncan-Lowey et al., 2020

toxins to the host, and induce reactive oxygen species production. Despite all these effects, these antimicrobials also prevent antimicrobial resistance and synergetics to antibiotics, which helps kill pathogenic organisms (Tariq et al., 2019; Zheng et al., 2020; Vaou et al., 2022), as shown in Figure 4.

Promote cell wall disruption and lysis

Cell disruption mechanism takes place *via* two approaches that are mechanical and non-mechanical approach (Figure 5). The detail of the antimicrobial mechanism is given in separate subsequent paragraphs. Firstly, compounds that functionalized this disruption are delineated as follows.

Phenolic compounds belong to the family of aromatics containing a hydroxyl functional group, which in reaction with the microorganisms disrupts their cell wall (Ganesan and Xu, 2017a, 2018). These aromatics are moved to the microbe's cell surface, and thus they disrupt their cell walls. Flavonoids are phenolic compounds that form a complex relationship with the bacterial cell wall and thus disrupt its structure (Ganesan and Xu, 2017b,c). Rutin, naringenin, sophoraflavanone, and tiliroside are the flavonoids that disrupt *S. aureus* and *S. mutans* microbes (Tsuchiya and Iinuma, 2000; Sanver et al., 2016). Terpenes contain isoprene that disrupts microbial membranes (Guimarães et al., 2019; Moghrovyan et al., 2019).

Induction of reactive oxygen species production

Reactive oxygen species are formed by the partial reduction of molecular oxygen that helps in antimicrobial activity and provides a defense mechanism against various microbes. The method of catechins involves augmentation of the production of oxidative stress that disrupts cell walls (Fathima and Rao, 2016; Van Acker and Coenye, 2017; Zou et al., 2017). Sources of reactive oxygen species, antioxidant defenses, and subsequent biological effects depending on the level of reactive oxygen species production are shown in Figure 6.

Inhibition of biofilm formation

Biofilms developed by bacteria are 100–1,000 times more resistant to the antimicrobial drugs (Kahaliw et al., 2017; Mishra et al., 2020). In some studies, it was indicated that flavonoids

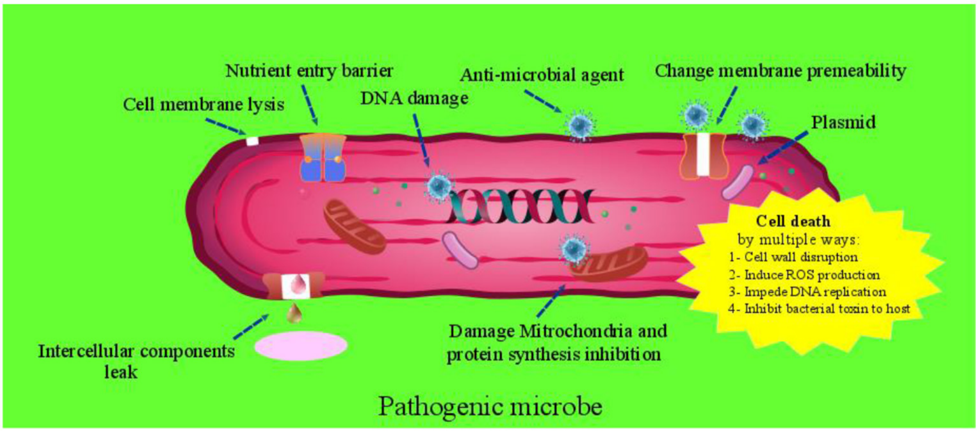

FIGURE 4
Antimicrobial mechanism of bioactive compounds.

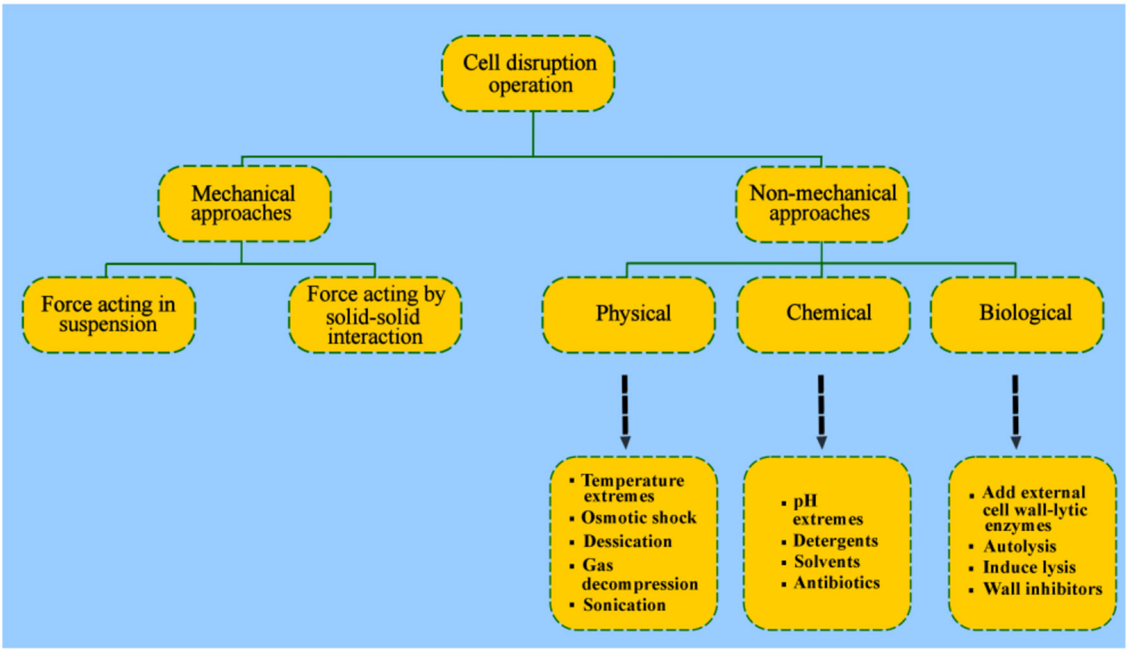

FIGURE 5
Antimicrobial mechanism by promoting cell wall disruption and lysis (Moo-Young, 2019).

aggregate the multicellular composites; thus, inhibiting bacteria growth. Flavonoids such as; (1) galangin, (2) isovitexin, (3) 3-O-octanoyl-epicatechin, and (4) 5, 7, and 40-trihydroxyflavanol cause the aggregation of *S. aureus* and *S. mutans*; therefore inhibiting their growths (Awolola et al., 2014; Rabin et al., 2015).

Inhibition of cell wall construction

The bacterial cell wall is the main target of any antimicrobial agent because the cell wall is the main source of biosynthesis of lipids, respiration and the transport mechanism. For all these functions, membrane integrity plays a major role in bacterial species, so the membrane disruption leads to bacterial death (Lazar and Walker, 2002; Klis et al., 2006; Reygaert, 2014).

Inhibition of bacterial toxins against the host

Catechins and some other flavonoids cause disruption of bacteria cell walls thus resulting in an inability to discharge toxins (Lee et al., 2011; Chang et al., 2019).

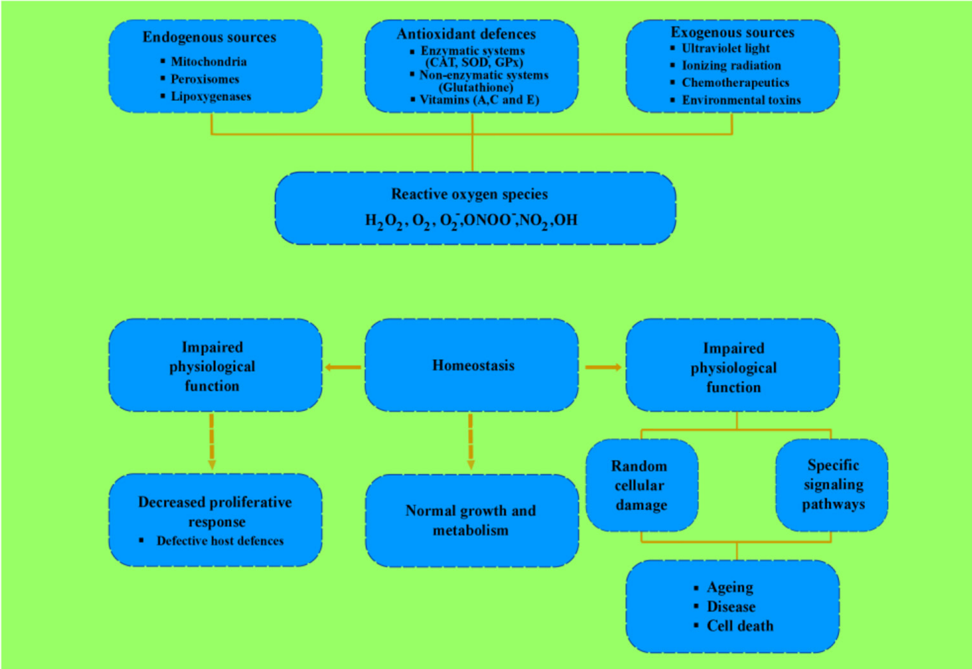

FIGURE 6
Endogenous and exogenous sources of ROS, antioxidant defenses, and biological effects that depends on ROS production.

Compounds such as catechins, pinocembrin, kaempferol, kaempferol-3-O-rutinoside, gallocatechin gallate, quercetin glycoside, genistein, and proanthocyanidins neutralize bacterial toxic factors initiating from *N. gonorrhoeae*, *V. cholerae*, *E. coli*, *B. anthracis*, *V. vulnificus*, *S. aureus*, and *C. botulinum* (Shahnazari et al., 2011; Ahmed et al., 2016).

Inhibition of energy synthesis

The production of energy or adenosine triphosphate (ATP) is important for the development of bacteria because it is the main source of a living system. The reaction of flavonoids such as isobavachalcone and 6-prenylapigenin with *S. aureus* results in disruption of the bacterial cell wall (Freudenberg and Mager, 1971; Kuete et al., 2011; Xie et al., 2015).

Inhibition of microbial DNA replication

Antimicrobial agents inhibit DNA replication thus stopping the further division of microbes. Moreover, bioactive compounds present in plants such as kaempferol, quercetin, apigenin, genistein, nobiletin, myricetin, chrysin, tangeritin, and 3, 6, 7, 30, 40-pentahydroxyflavone are DNA disrupters (Gotoh et al., 2008; van Eijk et al., 2017; Vijayakumar et al., 2018).

Antimicrobial mechanism of antimicrobial agents

Antimicrobial mechanisms of antimicrobial agents present in *Nigella sativa*, *Capsicum*, *Musa paradisiaca* L., and *Citrus limetta* are discussed below;

Antimicrobial mechanism of thymoquinone

Thymoquinone plays a major role in fighting against microbes. Basically, thymoquinone causes resistance in the biofilm formation in bacterial species because biofilm formation is the important activity of microbes in their virulence strategy. Thymoquinone also causes hindrance in the oxidative activity of microbes thus reducing the number of microbes (Harzallah et al., 2011; Forouzanfar et al., 2014; Khan, 2018; Fan et al., 2021).

Microbes cause many infectious diseases like AIDS, dengue, MERS, MRSA, and VRE, etc. Thymoquinone prevents the murine cytomegalovirus (CMV) replication in the liver and spleen of the infected mice. Medication with thymoquinone increases the number of $CD4^+$ T cells which plays a major role in building the immune system. Thymoquinone increases levels of serum interferon-γ (IFN-γ) and macrophages. Thymoquinone also fights against various fungal pathogens like *Candida albicans* and *Aspergillus fumigatus* (Khan, 2018; Wang et al., 2021; Qureshi et al., 2022).

Antimicrobial mechanism of melanin

Melanin constitutes a heterogenous group of phenolic polymers, which plays a major role in fighting against microbes. Melanin is an important antimicrobial agent which stops the proliferation of pathogens around the infected area. Melanin can fight against food pathogens; thus, contributes a major role in the food and health sector. Melanin also helped in antimicrobial drug discovery. Melanin extracted from hair and further dopped in metal ions demonstrated the potential of antibacterial activity (Mackintosh, 2001; Alviano et al., 2004; Nosanchuk and Casadevall, 2006; Eliato et al., 2021).

Antimicrobial mechanism of P-cymene

P-cymene is an important antimicrobial agent which is found in more than 100 plants and contributes to the food and medicine sector. P-cymene is used in the preparation of fungicides and pesticides. P-cymene affects the bacterial membrane, pH and ATP thus contributing to the file of antimicrobes. Studies reported that p-cymene inhibits the growth of *E. coli, L. monocytogenes, S. enterica*, *S. aureus, Vibrio parahaemolyticus*, and *Streptococcus mutans* (Kordali et al., 2008; Marchese et al., 2017; Miladi et al., 2017; Balahbib et al., 2021).

Antimicrobial mechanism of pinene

Pinene belongs to the group of terpenes and has antimicrobial properties. Pinene inhibits microorganisms by interfering in their metabolic process and modulation of gene (Tyc et al., 2017; do Amaral et al., 2020). Pinene has its antimicrobial activity (Dhar et al., 2014; Zhang et al., 2021) against *C. neoformans, C. neoformans phospholipase* and *esterase*, *C. Albicans*, *R. oryzae*, and MRSA (Silva et al., 2012; Kovač et al., 2015).

Antimicrobial mechanism of alkaloid

Alkaloids disrupt the cell membrane of microbes, inhibit cellular division, inhibit efflux pump, and inhibit biofilm formation thus act as a lead compound in the development of antimicrobial agents. Studies reported that alkaloids can kill *Pseudomonas aeruginosa, Streptococcus mutans, faecalis* and *pyogenes, Lactobacillus acidophilus, Candida albicans*, and *Staphylococcus aureus* (Orhan et al., 2010; Özçelik et al., 2011; Mittal and Jaitak, 2019; Othman et al., 2019; Zielińska et al., 2019; Huang et al., 2022).

Antimicrobial mechanism of limonene

Limonene belongs to the family of monoterpenes. Limonene by accumulating in the membrane of microbes causes disruption in it. Limonene is also one of the main causes of the dissipation of proton motive force in microbes. Limonene being an antimicrobial agent has a broad application in the food sector. Studies reported that limonene can kill gram-positive and gram-negative bacterial species. Limonene is also found to be effective against fungal pathogens (Cai et al., 2019; Han et al., 2019, 2020; Gupta et al., 2021).

Antimicrobial mechanism of camphene

Camphene is a lipophilic antimicrobial compound that by penetrating in cell membranes of bacterial species becomes the reason of cell death. Camphene inhibits microorganism by disruption in many cell activities, and the breakdown of the membrane thus cellular components and ions start leaking from the bacterial body. Also, camphene is the major source of depletion of ATP in bacterial species. Studies reported that camphene has antibacterial, antiviral, and antifungal activities (Zhou et al., 2016; Er et al., 2018; de Freitas et al., 2020; Hachlafi et al., 2021).

Conclusion

Microbial diseases are a major threat to the human population. The worldwide death rates due to microbes are: *Pseudomonas aeruginosa* 61%, *E. coli* 4.2%, *Staphylococcus epidermidis* 20%, *Candida albicans* 24%, *Shigella flexneri* 13.2%, *Sal. Typhimurium* 3 million each year, and *Vibrio alginolyticus* 50%. However, these death rates can be reduced by using antimicrobial agents on a regular basis. This review evaluated the antimicrobial effects of four plant resources- *Capsicum*, *Nigella sativa*, *Musa paradisiaca* L., and *Citrus limetta*. These natural resources contain thymoquinone, p-cymene, pinene, alkaloid, limonene, camphene, and melanin, which serve as antimicrobial agents by disrupting the cell membrane of microbes, inhibiting the cellular division and inhibiting the formation of biofilm in bacterial species, thus reducing the number of microbes. Soxhlet, hydro-distillation, solid-phase extraction (SPE), liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), pulsed electric field (PEF), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), and high-voltage electrical discharge are the various techniques used for extracting critical antimicrobial agents from *Capsicum*, *Nigella sativa*, peels of *Musa paradisiaca* L., and *Citrus limetta.* A brief description of all these techniques was given along with their advantages and disadvantages in order to ease the selection of appropriate technology for the extraction of naturally available antimicrobial compounds.

Author contributions

SB, WZ, MH, and AM equally contributed to the concept of the study, its framework, the coordination and activities of subgroups, and writing of the manuscript. MM, MG, MI, SS, SI, SU-R, and SK equally contributed to the activities of subgroups and the review of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Object detection and classification using few-shot learning in smart agriculture: A scoping mini review

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Smart agriculture is the application of modern information and communication technologies (ICT) to agriculture, leading to what we might call a third green revolution. These include object detection and classification such as plants, leaves, weeds, fruits as well as animals and pests in the agricultural domain. Object detection, one of the most fundamental and difficult issues in computer vision has attracted a lot of attention lately. Its evolution over the previous two decades can be seen as the pinnacle of computer vision advancement. The detection of objects can be done *via* digital image processing. Machine learning has achieved significant advances in the field of digital image processing in current years, significantly outperforming previous techniques. One of the techniques that is popular is Few-Shot Learning (FSL). FSL is a type of meta-learning in which a learner is given practice on several related tasks during the meta-training phase to be able to generalize successfully to new but related activities with a limited number of instances during the meta-testing phase. Here, the application of FSL in smart agriculture, with particular in the detection and classification is reported. The aim is to review the state of the art of currently available FSL models, networks, classifications, and offer some insights into possible future avenues of research. It is found that FSL shows a higher accuracy of 99.48% in vegetable disease recognition on a limited dataset. It is also shown that FSL is reliable to use with very few instances and less training time.

KEYWORDS

smart agriculture, object detection, computer vision, digital image processing, machine learning, few-shot learning

Few-Short Learning in agriculture

In the realm of computer vision, detecting small objects such as plants, leaves, weeds, fruits, and pests is a critical study topic. It is a technology that employs computer vision equipment to acquire photos to determine whether the obtained images include weeds, pests or even plant diseases (Lee et al., 2017). Computer vision-based object detection technology is currently being used in agriculture and has partially replaced traditional naked-eye identification (Liu and Wang, 2021). During the growth process, pests, and weeds frequently harm crops which leads to various diseases. They will become more dangerous if control is not done in a timely or effective manner. This will have an impact on crop yields and result in significant losses in agricultural income. People rely on their experiences while using traditional detection methods (Zhang S. et al., 2021).

Numerous agricultural plants, weeds, fruits, pests, and diseases have similar morphology, making it difficult to recognize them. Only judging by human eye observation is time-consuming, debilitating, biased, imprecise, and costly (Ashtiani et al., 2021). Nonprofessional agricultural employees will be harmed by their personal experiences since they will be unable to appropriately identify the species. As a result, pest control is an important aspect of agricultural productivity, and detecting agricultural pests is critical for agricultural development (Zhang S. et al., 2021).

As a result, an accurate detection technique is very desirable, and FSL is an approach that is precise in object detection. FSL learning is a sub-area in machine learning. FSL is a sort of machine learning problem (defined by E, T, and P), in which E, Experience, comprises a small number of supervised instances for T, Task, measured by P, Performance (Wang et al., 2020). FSL is used to quickly train the model on a little amount of tagged data (Fei-Fei et al., 2006). The fundamental concept behind FSL is comparable to how humans learn: it makes use of previously learned knowledge to pick up new skills while minimizing the quantity of training data needed. Research findings (Duan et al., 2021) demonstrate that FSL can address several machine learning's drawbacks, such as the need for huge datasets to be collected and labeled, by dramatically lowering the quantity of data necessary to train machine learning models. Due to the small number of datasets, the data are divided into two splits [train and test (without validation) or train and validation (without test)] in many types of research in the field of artificial intelligence, which can be a weakness. The data set is divided into two subsets because, in small data sets, an additional split could result in a smaller training set that is more susceptible to overfitting (Ashtiani et al., 2021).

A framework put forth by Fink (2004) allows object classifiers to be learned from a single example, which drastically reduces the amount of data gathering required for image classification tasks. Additionally, FSL enables the training of models that are appropriate for a few uncommon circumstances (Duan et al., 2021). A good example is the significant advancements in drug development that have been made as a result of the most recent FSL research advancements (Jiménez-Luna et al., 2020). Clinical biological data for a given drug are extremely scarce when predicting whether it is harmful, which frequently makes use of classic machine learning algorithms difficult.

FSL is widely used for scene classification (Alajaji et al., 2020), text classification (Muthukumar, 2021), image classification (Li X. et al., 2020) and image retrieval (Zhong Q. et al., 2020). Even though software and computational technologies have long been utilized for detection in agriculture, FSL's capacity to identify them with limited training data makes them suitable for the task. This is ground-breaking because, they can be detected under various conditions of lighting, orientation, and background (Nuthalapati and Tunga, 2021).

FSL has been proven in agriculture to be an effective method for plant disease identification (Argüeso et al., 2020). Recent FSL approaches in agriculture are mainly used for plant disease detection (Wang and Wang, 2019; Li and Chao, 2021), fruit detection and classification (Janarthan et al., 2020; Ng et al., 2022), leaf identification and classification (Afifi et al., 2020; Jadon, 2020; Tassis and Krohling, 2022) and pest detection (Li and Yang, 2020, 2021; Nuthalapati and Tunga, 2021) using the meta-train set which is subsequently used to build embeddings for the samples in the meta-test set.

This article reviews the limitations of deep learning, advantages of FSL, types of FSL networks, their applications in the agricultural sector and their corresponding findings.

Limitations of deep learning

In agriculture, deep learning-based automatic classification of several categories is a hot topic of research (Too et al., 2019; Li Y. et al., 2020). Yet, in general, people still lean on non-automated classification by experts for the classification of distinct species. This is due in part to the fact that deep learning networks based on Convolutional Neural Networks (CNNs) demand thousands of labeled examples per target category for training, and labeling samples on such a large scale necessitates domain experts (Krizhevsky et al., 2017).

Furthermore, following training, the number of categories that a trained CNN-based model can recognize remains constant. To increase the number of categories that the network can recognize, it must be fine-tuned by adding fresh examples from other classes (Chen et al., 2019). Fine-tuning is a transfer learning approach which is used to benefit from the pre-trained network by adjusting the parameters to the data set. Due to the pre-established weights in a pre-trained network, fine-tuning is quicker than training from start (Ashtiani et al., 2021). Also, adequate data is required during training to avoid the network from overfitting (Gidaris and Komodakis, 2018) whereas humans can acquire new skills with little to no guidance using one or a few examples only (Lake et al., 2011).

Advantages of Few-Shot Learning

Humans can generalize new information based on a small number of examples, whereas artificial intelligence typically needs thousands of examples to produce comparable results (Duan et al., 2021). Researchers sought to create a machine learning model that, after learning a significant amount of data for some categories, could quickly learn a new category with only a few sample data, drawing inspiration from humans' rapid learning abilities. This is the issue that FSL seeks to address. This task only offers a small number of observable examples, much like the production of character samples (Lake et al., 2015).

FSL has lately received a lot of attention as a way for networks to learn from a few samples. FSL tries to solve the challenge of classification with a small number of training examples, and it is gaining traction in a variety of domains (Lake et al., 2011). In FSL, there are two sets of labeled image data which are meta-train and meta-test, with image classes that are mutually exclusive in both sets (Argüeso et al., 2020). The goal is to develop a classifier on the visual classes in the meta-test set that can classify a particular query sample even with very few labeled examples using the data in the meta-train set and learn transferrable information (Altae-Tran et al., 2017).

Additionally, cross-domain FSL is defined as when the domain of visual classes in the meta-train set differs from the domain of visual classes in the meta-test set (Argüeso et al., 2020). One of the primary issues in cross-domain FSL is feature distribution differences between domains. Likewise, both the meta-train and meta-test sets in mixed-domain FSL contain classes from many domains, whereas single-domain FSL (Li and Yang, 2021) only contains instances from a single domain.

According to research (Duan et al., 2021), FSL can learn about uncommon circumstances where it is challenging to find information about supervision by combining one or a few examples with previously learned knowledge. This can solve the issue of the machine learning algorithm's need for thousands of supervised examples to guarantee the model's generalizability. Additionally, FSL can help expedite the training cycle and lower the high cost of data preparation (Parnami and Lee, 2022).

Types of networks in Few-Shot Learning

According to the traditional paradigm, an algorithm learns whether task performance gets better with practice when given a specific task. The Meta-Learning paradigm consists of several tasks. An algorithm that is learning to determine whether its efficiency at each task increases with practice and the number of tasks is called a meta-learning algorithm (Chen et al., 2021). Numerous Meta-Learning techniques have been released by researchers in recent years to address FSL classification issues. Metric-Learning (Yang and Jin, 2006) and Gradient-Based Meta-Learning (Khodak et al., 2019) methods can be used to categorize them all into two main classes.

Metric-learning is the term used to describe the process of learning a distance function over objects (Chen et al., 2021). Algorithms using metrics learn to compare data samples in general. They categorize query samples for an FSL classification problem according to how similar they are to the support samples. A base-learner and a meta-learner are created for the gradient-based technique. A base-learner is a model that is initialized and taught within each episode by the meta-learner, whereas a meta-learner is a model that learns across episodes (Cao et al., 2019). Table 1 shows the meta-learning algorithms with its category respectively.

One of the most well-liked meta-learning algorithms, Model-Agnostic Meta-Learning (MAML) has made significant advancements in the field of meta-learning research. Furthermore, meta-learning is a two-part learning process where the model first prepares to learn new tasks and then actually learns the new task from a few samples in the second stage (Yavari, 2020). The general goal of MAML is to identify suitable starting weight values so that the model can quickly pick up new tasks.

The first Metric-Learning method created to address FSL issues was Matching Networks (Chen et al., 2021). When using the Matching Networks approach to resolve an FSL job, a large base dataset is required (Li X. et al., 2020). This dataset is divided into episodes. Every image from the query and support sets is given to a CNN, which produces embeddings for each one. The SoftMax of the cosine distance between each query image's embeddings and the support-set embeddings is used to classify each image. It also uses Long Short-Term Memory (LSTM) for testing and Bidirectional Long Short-Term Memory (biLSTM) for training (Ye et al., 2020). The resulting classification's Cross-Entropy Loss is backpropagated through the CNN. Matching Networks can learn to construct picture embeddings in this way. Matching Networks can categorize photographs using this method without having any special prior knowledge of classes (Vinyals et al., 2016). Simply comparing several instances of the classes is used for everything.

Prototypical Networks learn a metric space in which each class may be classified by computing distances between prototype representations (Yavari, 2020). They represent a more straightforward inductive bias, which is advantageous in this low-data environment. The notion behind Prototypical Networks is that each class has an embedding in which points cluster around a single prototype representation (Pan et al., 2019). To accomplish so, a neural network is used to learn a non-linear mapping of the input into an embedding space, and a class's prototype is taken to be the mean of its embedding space support set. The next step is to classify an embedded query point by simply identifying the closest class prototype (Ji et al., 2020).

Another straightforward and efficient technique for both one-shot and FSL that adheres to the metric-learning paradigm is the Relation Network (Yavari, 2020). Relation Network uses an embedding function f to extract the features from the inputs, just like the Prototypical and Siamese Networks (Sung et al., 2018). As an embedding function, CNN is utilized because the inputs are images. The Relation Network includes a relation function in addition to the embedding function. This relation function evaluates how closely the query sample is connected to the various classes in the support set (Sun et al., 2018).

TABLE 1 Types of meta learning algorithms.

Networks	Category	Embedding function for testing	Embedding function for training
Model-agnostic meta learning (Wang and Wang, 2019)	Gradient-based meta learning	Deep neural network	Deep neural network
Matching network (Wang and Wang, 2019)	Metric learning	CNN, LSTM	CNN, biLSTM
Prototypical network (Yavari, 2020)	Metric learning	CNN	CNN
Relation network (Yavari, 2020)	Metric learning	CNN	CNN
Siamese network (Yavari, 2020)	Metric learning	CNN	CNN

A Siamese Network is made up of two networks that receive different inputs but are connected at the top by an energy function (Yavari, 2020). This function calculates a metric between each side's highest-level feature representation. The dimensions of the twin networks are inextricably linked (Dong and Shen, 2018). The network's structure is duplicated in both the top and bottom parts, resulting in twin networks with shared weight matrices at each layer. The Siamese Network's goal is to evaluate two photos and determine whether they are similar (Melekhov et al., 2016). The process for comparing a Siamese network is quite straightforward.

Other methods for small data training

There are also methods other than FSL for small data training. Knowledge may be successfully exploited in sophisticated applications by being appropriately organized and represented in knowledge graphs (Chen et al., 2020). Knowledge graphs can efficiently manage, mine, and organize knowledge from massive amounts of data, enhancing the quality of information services and delivering more intelligent services to users. It is used to store connected descriptions and details about things like events, objects, actual circumstances, and theoretical or abstract ideas (Pujara et al., 2013). This approach simultaneously encodes semantics underlying the data collection in addition to serving as data storage. The arrangement and structuring of significant data points from the data set to integrate information gathered from multiple sources is the main purpose of the knowledge graphs model. Labels are added to a knowledge graph to associate certain meanings. Nodes and edges are the two basic parts of a graph (Wang et al., 2017). Nodes are made up of two or more objects, and edges show how they are related to one another.

By using enough prior knowledge from related fields to complete new tasks in the target domain, transfer learning simulates the human visual system despite the size of the data set (Shao et al., 2014). In transfer learning, the source domain and the target domain are two different sorts of domains that can both benefit from the training and testing data (Weiss et al., 2016). The source domain contains training examples, which are under a different distribution from the target domain data, and the target domain contains the testing instances, which are the classification system's task. For a transfer learning task, there is typically just one target domain, although there may be one or more source domains (Torrey and Shavlik, 2010).

Another popular method is self-supervised learning (SSL). SSL has becoming more common since it can save on the expense of annotating huge datasets (Jaiswal et al., 2020). It has the ability to employ the learnt representations for numerous downstream tasks and use self-defined pseudo labels as supervision. Particularly, contrastive learning has lately taken center stage in self-supervised learning for computer vision, natural language processing (NLP), and other fields. It attempts to push embeddings from various samples away while trying to embed augmented copies of the same sample closely together (Zhai et al., 2019).

Methodology

A literature search has been carried out by querying Google Scholar, IEEE Xplore, ScienceDirect, SpringerLink, ACM Digital Library and Scopus. According to studies, Google Scholar's reach and coverage are greater than those of similar academic websites. Then, using exclusion criteria, a small number of databases is chosen to search for pertinent papers. The search was conducted using the terms "few shot learning", "smart agriculture", "object detection", "few shot classification", "one shot learning", "low shot learning", "Meta-Learning", "Prototypical Network", "Siamese Network", "MAML", "Relation Network", and "Matching Network".

The search was also limited to recent research papers in the years 2010 and above. Repeated things between search engine results were searched by employing a straightforward script based on the edit distance between article titles. Around 150 articles were returned by all the search engines with 58 articles in IEEE Xplore, 50 articles in ScienceDirect, 21 articles in Google Scholar, 15 articles in SpringerLink, and six articles in Scopus. The resulting papers were very diverse, and most of them fell outside the purview of the review. As a result, studies that had been published and that dealt with FSL in agriculture and reported noteworthy findings considering recent literature have been chosen. The search was restricted to keywords, titles, and abstracts.

Exclusion criteria were used to screen out works that had nothing to do with the literature review. The following standards are disregarded in this analysis: (a) The ensuing articles have nothing to do with FSL in agriculture, (b) Articles that are not written in English, (c) Articles that are duplicated in the databases, (d) Articles that do not have significant results and problems. This filtered to 30 articles and during the process, references of the selected papers were also looked to find other works that fit the inclusion criteria. Finally, the resulting articles were organized by considering the models and types of objects in the agriculture field.

Results and discussion

FSL was first used in the field of computer vision, where it produced promising results in picture classification. It does not require large samples during training, which reduces the time and cost of obtaining samples. As a result, it is now being welcomed in many fields such as smart agriculture (Yang et al., 2022). FSL has shown compelling results in smart agriculture such as plant disease classification, plant leaf classification and plant counting. Early diagnosis of plant diseases is crucial for preventing plagues and mitigating their consequences on crops.

Deep learning is used to create the most accurate automatic algorithms for identifying plant diseases using plant field pictures. Traditional machine learning and deep learning techniques, on the other hand, have certain drawbacks, one of which is the requirement for large-scale datasets for models to generalize well (Wang et al., 2020). These methods necessitate the collecting and annotation of huge image datasets, which is usually impossible to achieve due to technological or financial constraints. As a result, new strategies were presented with the goal of developing models that generalized well with fewer data. As a result, meta-learning approaches (Hospedales et al., 2021) and FSL methods, were proposed. The Table 2 shows the results of the findings.

Research on identification has begun over two decades. Deep learning was used in these studies, and they were a huge success. However, there are a few challenges that deserve greater attention and research, including the demand for a big training set, training time, CNN architecture, pre-processing, feature extractions, and performance evaluation measures. Some of the experiments will not work in the wild due to camouflaging of the backgrounds (Liu et al., 2019; Kuzuhara et al., 2020; Yao et al., 2020).

To address these problems, authors have utilized machine learning in the identification of pests as this study makes the machine to think like humans. Machine learning has shown satisfactory results in most studies (Brunelli et al., 2019; Tachai et al., 2021; Zhang H. et al., 2021). However, machine learning also uses CNN feature extractions for training which shows improvement. Classifiers such as Support Vector Machine (SVM) is the best classifier that enhances the accuracy (Kasinathan et al., 2021).

To fill the gap between large training samples and time consumption, FSL was implemented. FSL has become popular in the computer vision field in both classification and detection. FSL was introduced very recently, and the results generated by this meta-learning approach are favorable. In the smart agriculture sector, FSL is widely used in plant disease identification (Wang and Wang, 2019; Li and Chao, 2021), plant counting (Karami et al., 2020), leaf classification (Afifi et al., 2020; Jadon, 2020; Tassis and Krohling, 2022) and fruit ripeness classification (Janarthan et al., 2020; Ng et al., 2022). The results for these experiments have exceeded 90%.

For plant leaf classification using the Siamese network, the accuracy of 95.32%, 91.37% and 91.75% were achieved for Flavia, Swedish and Leafsnap respectively (Wang and Wang, 2019). For maize plant counting, the overall precision was above 95% (Karami et al., 2020). For plant disease classification using triplet loss, the accuracy was 91.4% and using the Siamese network the accuracy was 90.6% (Argüeso et al., 2020). In vegetable disease recognition 99.48% accuracy was reached (Wang et al., 2021). For pest detection, have utilized a prototypical network and triplet loss with a terminal realization for cotton pests. They used two datasets for comparison and achieved 95.4 and 96.2% respectively (Li and Yang, 2020; Nuthalapati and Tunga, 2021).

However, some FSL techniques show lower accuracies due to their limitations. For fruit ripeness classification, they achieved 75% accuracy for only five samples (Ng et al., 2022). Another plant disease recognition has achieved an accuracy of around 55% for zero-shot (Zhong F. et al., 2020). For pests, one study achieved 60.3% (Nuthalapati and Tunga, 2021). For animal detection, the authors achieved 55.61 and 71.03% for 1 and 5 shots respectively due to no semantic annotations in testing (Zhang H. et al., 2021). Another bird detection study achieved 60.19 and 75.75% for 1 and 5 shots respectively (Liu et al., 2019).

Even though some research has lower accuracies, they are still satisfactory because FSL only uses very less instances in the training of 1, 2, 3, 5, and 10 images for detection and classification (Zhong F. et al., 2020). This proves that FSL is giving a good result with very less training samples compared to CNN which requires more training samples and training time.

Main findings and ways forward

Deep learning and machine learning is the process of utilizing algorithms to direct a computer in building a suitable model based on existing data and then using that model to evaluate novel situations. The models' applicability are significantly constrained by the massive amounts of labeled data that are often needed to train it because it has multiple parameters. Large amounts of tagged data are frequently

TABLE 2 Studies of few-shot learning in smart agriculture.

References	Data source	Techniques	Accuracy	Purpose	Limitation
Wang and Wang (2019)	Flavia Dataset; Swedish Dataset; Leafsnap Dataset	Siamese Network; Simultaneous Two-way Convolutional Neural Network; Spatial Structure Optimizer (SSO); K-Nearest Neighbor (KNN)	Flavia = 95.32%; Swedish = 91.37%; Leafsnap = 91.75%	To handle a leaf classification problem with a small sample size using Siamese Network	Has to improve the generalization ability of the model
Karami et al. (2020)	Maize Plant Dataset	Modified CenterNet Architecture	>95%	To identify and count maize plant by using a smaller number of images in a field using FSL	Adapting generative adversarial network (GAN) in future
Ng et al. (2022)	Fruit Dataset	Meta-learning paradigm; Few-shot classification	>75%	To classify new varieties of fruits with only a few training examples	Not stated
Argüeso et al. (2020)	PlantVillage Dataset	CNN; SVM Classifier; Siamese Network; Few-shot and baseline transfer learning; Root Mean Square optimizer (RMSprop)	Triplet Loss; Accuracy = 91.4%; Precision = 93.8%; Recall = 92.6%; F-Score = 92.3%; Siamese; Accuracy = 90.6%; Precision = 93.8%; Recall = 93.1%; F-Score = 91.2%	To classify plant diseases to overcome unfeasibility of deep learning	Convergence problems
Zhong F. et al. (2020)	CUB Dataset; AWA2 Dataset; APY Dataset	Conditional Adversarial Autoencoders (CAAE); Kullback-Leibler (KL) Divergence Penalty	Zero Shot; Unseen = 55.4%; Seen = 51.6%; Harmonic = 53.4%	Recognition of Citrus aurantium L. diseases using few and zero shot learning	Only uses a discriminator to replace KL-divergence in variational autoencoders
Li and Chao (2021)	PlantVillage Dataset	Semi-supervised few-shot learning	5-shot = 90%; 10-shot = 92.6%	To resolve plant leaf disease recognition problem using semi-supervised FSL	Did not consider wrong labels
Wang et al. (2021)	Self-Collected Dataset	Image text collaborative representation learning (ITC-Net)	Accuracy = 99.48%; Precision = 98.90%; Sensitivity = 98.78%; Specificity = 99.66%	Recognition of vegetable illness in complicated backgrounds	The feature spaces of image and text are still independent
Jadon (2020)	Mini-Leaves Diseases Dataset; Sugarcane Dataset	Stacked Siamese Matching (SSM) Net	Mini Leaves = 92.7%; Sugarcane = 94.3%; Comparison with VGG16; Mini Leaves = 0.91; Sugarcane = 0.90	Mini-leaves diseases and sugarcane illness identification using combined methods of Siamese and Matching networks	Not stated
Hu et al. (2019)	Tea Leaf Disease Image Dataset	Conditional deep convolutional generative adversarial networks (C-DCGAN); Support vector machine (SVM)	90%	Tea leaf disease identification with low shot learning	Low generalization performance
Janarthan et al. (2020)	Citrus Fruits and Leaves Dataset	Deep Siamese Network	95.04%	To create a classification strategy for diagnosing citrus diseases	Not Stated
Afifi et al. (2020)	PlantVillage Dataset; Coffee Leaf Dataset	Triplet network; Deep adversarial metric learning (DAML)	99%	Automatic identification of plant diseases with limited data	Focusing on the full image instead of focusing on the affected part

(Continued)

TABLE 2 (Continued)

References	Data source	Techniques	Accuracy	Purpose	Limitation
Tassis and Krohling (2022)	Leaf Dataset; Symptoms Dataset	Prototypical networks; Triplet networks; feature extraction	1st Experiment = 96.03%; 2nd Experiment = 96.72%; 3rd Experiment = 93.25%	FSL in classification and severity assessment tasks with biotic stressors in coffee leaves	During training, there is a lack of performance evaluation of the approaches in unobserved classrooms
Li and Yang (2020)	National Bureau of Agricultural Insect Resources (NBAIR) Dataset; Li's dataset of natural scenes	Prototypical Network; CNN; Triplet loss; Terminal realization	95.4% (National Bureau of Agricultural Insect Resources dataset); 96.2% (Li's dataset of natural scenes)	Few shot cotton pest recognition and terminal realization	Not stated
Nuthalapati and Tunga (2021)	Plant and Pests (PP) dataset; PlantVillage dataset; Plants in Wild (PiW) dataset; Cross dataset	Few-shot learning using transformer	Plant and Pests = 60.3%; PlantVillage = 88.5%; Plants in Wild = 93.3%; Cross = 86.1%	To classify diverse pests, plants, and illness	Not stated
Li and Yang (2021)	PlantVillage Dataset	Few-shot classification; CNN	Single domain,; Ntrain = Ntest = 3 = 90.4%,; Ntrain = Ntest = 5 = 82.5%.; Cross domain 1,; Ntrain = Ntest = 3 = 86.5%,; Ntrain = Ntest = 5 = 81%.; Cross domain 2,; Ntrain = Ntest = 3 is 54.8%,; Ntrain = Ntest = 5 is 43.9%.	Task-driven meta-learning few shot classification for pests and plants and to investigate single domain and cross-domain classification	Not stated
Zhang H. et al. (2021)	MiniImagenet Dataset; CUB-200-2011 Dataset; Flower102 Dataset	Absolute-relative Learning	1-shot = 55.61%; 5-shot = 71.03%	Absolute-relative learning paradigm in supervised and unsupervised FSL	Only used semantic annotations as labels in training, and do not use them during testing
Liu et al. (2019)	ImageNet Dataset	Few-Shot Unsupervised Image-to-Image Translation (FUNIT); Generative Adversarial Networks (GAN)	Animal Face; 1-shot = 82.36%; 5-shot = 96.05%; North American Birds; 1-shot = 60.19%; 5-shot = 75.75%	Few-shot unsupervised image-to-image translation of animal faces, birds, flowers, and foods	FUNIT fails to achieve translation when the look of novel object classes differs considerably from that of the source classes

exceedingly expensive, challenging, or even impossible to obtain. FSL is a crucial component of the development of machine learning since it is a crucial question if a good model can be created by training with only a minimal amount of labeled data. It may be applied to learn uncommon situations using a limited collection of characteristics, which is a method that can successfully address the issue of dataset imbalance in intrusion detection.

In recent years detection and classification in the agricultural domain has received a lot of attention. Beginning with deep learning and machine learning, they are showing excellent results in identifying the objects. This approach is very useful for limited datasets and time for detection as well as classification of various things including plant diseases and pests. Most of the authors used the current state of the art in FSL such as the Siamese network, Prototypical network, and Model-Agnostic Meta Learning network.

Most of the FSL approaches show favorable results of more than 90%. One research has reached 99.48% accuracy in vegetable disease detection using a self-collected dataset. Even though the accuracy is very excellent in this research, the authors stated that the feature spaces of image and text are still independent, and it needs to be improved in the future. Triplet network and deep adversarial metric learning have also given accuracies of 99% with full coffee leaf images. Besides FSL, one shot learning has also shown a very satisfying result of 95.32% using the Siamese network. The low generalization ability of the model is an issue in this research and the gradient descent in the training must be stopped at the appropriate point as well as adding a regularization term to the error function to smooth out the mappings.

Most of the results of plant datasets have higher accuracies than animal datasets. However, pest detection using a prototypical network has achieved 96.2% accuracy. Another pest detection only achieved 60.3% due to camouflaging images in the background which can be improved by focusing on the insects only. The least accuracies that have been obtained are with animal detection, 55.61% for one shot. However, when five shots were used, the accuracy increased to 71.03%. This proves that when more instances are used, the accuracy increases. Despite that, Few-Shot Unsupervised Image-to-Image Translation (FUNIT) has produced an accuracy of 82.36% for one shot and 96.05% for five shots using animal faces. For birds, an accuracy of 60.19% for one shot and 75.75% for five shots was obtained.

According to studies reviewed here, FSL can significantly boost the detection performance on small datasets. The popularity of FSL for smart farming applications is increasing rapidly due to the straightforward implementation yet highly promising results obtained in agricultural applications.

Author contributions

NR: writing—original draft and investigation. JT: writing—review and editing, supervision, and conceptualization. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

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Development of functional yogurt by using freeze-drying on soybean and mung bean peel powders

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Introduction: Plant-based yogurt has earned much interest in current times due to the rising demand for milk substitutes, which is tied to ethical and health needs.

Methods: Freeze-drying impact on soybean peel powder (SPP) and mung bean peel powder (MPP) and their use in creating functional yogurt at various concentrations was checked. In functional yogurt, total flavonoid content (TFC), total phenolic content (TPC), antioxidant activity and chemical profile are checked.

Results: The maximum concentration of TPC was 4.65±0.05 (mg GAE/g), TFC was 1.74±0.05 (CE mg/g) and 82.99 ± 0.02 % antioxidant activity was calculated in sample T^6, having the highest concentration of SPP, which was substantially more significant than the treatment samples containing MPP. Sensory attributes of the yogurt samples were analyzed, which indicated a decrease when SPP and MPP values increased when introduced at 3 or 6 % of an optimum level. There was no notable loss of the sensory profile compared to the control group. The results were found to be significant at $p < 0.05$. The freeze-dried SPP had the complete chemical composition compared to MPP except for ash and fiber content.

Discussion: The physicochemical profile of the treatments of functional yogurt had a linear proportional connection in the percentage of both powders in the meantime. When both the dry level of powders increased, the protein and fat levels decreased. In the food industry, the freeze-dried soybean peel and the peel of mung bean can be utilized in functional yogurt as a source of bioactive components.

KEYWORDS

freeze-drying, soybean peel, mung bean peel, bioactive compounds, antioxidant activity

1. Introduction

Phytochemicals, dietary fiber, and other essential components abound in agro-industrial waste. This trash is frequently burnt or abandoned, even though it contains numerous valuable nutrients (Thakur, 2020; Khalid et al., 2022a,b,c). One of the essential industrial wastes, soybean has one of the highest concentrations of dietary fiber, bioactive substances, and other critical elements (Sagar et al., 2018). The phrase "food by-products" has gained popularity throughout time. The word clarifies that biological waste may be appropriately processed and transformed into more valuable compounds for the market (Kour et al., 2019; Trivedi et al., 2020; Ali et al., 2022a).

Because of their nutritional qualities (polyunsaturated fatty acids, dietary fibers, and high-quality proteins) and their critical role in lowering the risk of chronic illnesses, soy and mung bean products are becoming a more significant component of the human diet (Kumar and Pandey, 2020; Ali et al., 2021a; Khalid et al., 2022d). Soy products may promote bone health, alleviate menopausal symptoms, and lower the risk of heart disease, type 2 diabetes and cancer (Pabich and Materska, 2019; Ali et al., 2021b, 2022b; Aziz et al., 2022). But for the sector in question, disposing of the by-products of soy and mung bean food production is a significant challenge. Soybean has various drawbacks, such as an unpleasant bean flavor for particular operations and flatulence caused by their raffinose and stachyose concentration (Hachmeister and Fung, 1993; Li et al., 2022). These two beans contain different essential components like mung bean (25–28% protein) and a resource of bioactive components such as polysaccharides, phenolic acids, organic acids, and flavonoids (Kim et al., 2016).

Despite being somewhat more expensive than other drying procedures, freeze-drying yields the finest quality food product attainable (Ishwarya et al., 2015; Ali et al., 2022a). Microwave freeze-drying and atmospheric freeze-drying are two other kinds of freeze-drying that are often employed in the food business (Duan et al., 2016; Ahmed et al., 2022; Maqbool et al., 2022). Due to the elimination of water content in materials by sublimation in both ways, the product quality of these two drying techniques is comparable to that of freeze-drying. Even though there has been a lot of scientific studies done on drying methods, studies have shown that freeze-drying is more efficient (Abdelwahed et al., 2006; Ahmed et al., 2022; Ahmad et al., 2023).

Products that have been freeze-dried retain a large portion of their original flavor and phytochemical qualities (Mustafa et al., 2019). They are also found to be both exceedingly light and crunchy. Since each organic substance (food) has unique features and various requirements, no specific moisture removal procedure is suitable for all kinds of food products (Malode et al., 2021; Khalid et al., 2022a; Manzoor et al., 2022). There is an urgent need to cease squandering and burning these nutrient-dense by-products that can be easily incorporated into new goods to compensate for nutritional shortages in present goods and extend the shelf life (Hansbro, 2023).

This research aims to verify that peel powders from soybean and mung beans may be used to create fermented functional foods high in fiber, like yogurt. The bioactive potential of the freeze-dried soybean and mung bean peel powder has been investigated at several phases of the yogurt production process.

2. Materials and methods

2.1. Raw material procurement

Raw milk, soybeans, and mung beans were purchased from the local market of Lahore city. To prevent contamination, milk, soybean and mung bean were placed into plastic bags. Additionally, the raw materials were sent to the Department of Food Science's fruits and vegetable laboratory at Government College University Faisalabad for additional evaluation.

TABLE 1 Optimized conditions of freeze dryer.

Factors	SP	MP
Freezing temperature (°C)	−28	−78
Blanched [100 (°C)]	+	+
T_{sh} (°C)	90	90
RM (%)	2.3	1.2
P_{kch} (mbr)	1.1	1.3

SP, soybean peel; MP, mung bean peel.

2.2. Handling of raw material

The laboratory was supplied with milk, mung beans, and soybeans for processing.

First, distilled water was used to wash the soybean and mung bean properly. While removing the peel from the soybean and mung bean, the milk container was stored in a 7°C refrigerator.

2.3. Drying of soy and mung bean peel by freeze-drying

With some necessary adjustments, the freeze-drying method, as suggested by Fischer et al. (2011) was used to dry soy and mung bean peel. This was accomplished using a laboratory freeze-dryer (ALPHA 1-2 LD Plus, Christ, and USA). As indicated in Table 1, several parameters, including freezing temperature, Pkch (mbr) vacuum pressure, Tsh temperature, and residual moisture level were established for wet soy and mung bean peel freeze-drying. In a freeze-dryer, wet Soy and mung bean peel was also transported for drying. The powders were gathered after drying and kept for further examination at room temperature in aluminum pouches. Soy and mung bean peel were dried, then crushed into a fine powder for future research and the creation of functional yogurt.

2.4. Product development

As stated in Table 2, SPP and MPP were measured, then added to the pasteurized milk but first the milk was inoculated with cultures having *Streptococcus thermophiles and Lactobacillus bulgaricus*. The mixture was mixed at 46°C and let 12–24 h for fermentation.

2.5. Chemical composition

By measuring the moisture content of dry powder 2 g, precisely., drying it at 100 5°C for roughly 3 h, and then weighing it again, the moisture content was determined. The Kjeldahl and Soxhlet equipment further evaluated the protein and fat levels. Additionally, a 2 g sample was heated to 400°C for 3 h in a muffle furnace under controlled circumstances to determine the sample's ash composition. This particular endeavor also looked into the

TABLE 2 Treatment table.

Treatments		Levels (%)
T_0	*Control*	0%
T_1	*Soybean peel (dried)*	3%
T_2		6%
T_3		9%
T_4	*Mung bean peel powder (dried)*	3%
T_5		6%
T_6		9%
T_7	*SPP + MPP*	2.5+2.5%

SPP, soybean peel powder; MPP, mung bean peel powder.

crude fiber. Meanwhile, the following expression was used to compute nitrogen- or carbohydrate-free extracts:

$$\%Carbohydrates\ (NFE) = 100 - (Fat + Protein + Moisture + Ash + Crude\ Fiber).$$

2.6. Yogurt's bioactive characterization

2.6.1. Extraction of sample

Utilizing the 9,676.8 g in a KMF grinder (grinder used to grind cereals like beans), freeze-dried soy and mung bean peel samples were reduced to powder. Before further extraction, prepared grounded samples were stored at 40°C in sterile bags to avoid contamination. To make the extracts, ethyl alcohol, methyl alcohol, and water were used. A flask with 100 mL of ethyl acetate was precisely filled with 0.5 g of dry material and then swirled for 3 h at 20°C. A Harrier 18/80 refrigerated centrifuge (MSE, UK, SANYO) was used to spin the mixture at 9,676.8 g for 30°C. Furthermore, the supernatant was filtered using Whitman filter paper (No. 1, 155 mm). The obtained extracts were stored at 4°C for further testing.

2.6.2. Antioxidant activity

Antioxidant activity was evaluated using the technique (2,2-diphenyl-1-picryl hydroxyl) described by Fischer et al. (2011), with specified minor adjustments. For this, precisely 15 ml of the extracts were put into a test tube along with 750 mL of the 0.1 mM DPPH and 735 mL of methanol solution. Everything was well homogenized until the section was completely dissolved in the methanol. The mixture was stored in the dark for exactly 30 min to avoid exposure to light. The absorbance at 517 nm was measured using a UV-visible spectrophotometer (Madison, ermo Scientific Technologies, WI, USA). The findings were presented as mg of extracts corresponding to mM ascorbic acid (AA).

2.6.3. Total phenolic content

According to Donkor et al. (2007), using the Folin-Ciocalteu technique, the TPC (total phenolic content) of the extracted materials was evaluated. This was done by carefully adding 250 mL of the Folin-Ciocalteu reagent, 750 mL of Na_2Co_3 (1.9 M), and 70 mL of the generated extracts to a test tube with a 10 mL capacity. A volume of precisely 5 mL was created by adding distilled water. This volume was then combined for roughly a minute in a vortex mixer before being kept in the darkness. The absorbance was estimated *via* a spectrophotometer (Surrey, England's Ermo-Spectronic) at a wavelength of 765 nm. The result was represented in mg dry solids of gallic acid equivalents (GAE).

2.6.4. Total flavonoid content

Using a procedure stated by Xu and Chang (2009), TFC was determined for mung bean peel and freeze-dried soybean extract. To prepare the extract, 4 mL distilled water in a test tube of 10 and 1 mL of the obtained extract were used. After this, 0.3 mL of 5% sodium nitrite was added to the test tube. After 5 min, 0.3 mL aluminum chloride of 10% was added to the test tube. 2 mL of 1 M sodium hydroxide has been added to the test tube and thoroughly homogenized. Now, 2.4 mL of clean water was quickly used to dilute the test tube, and it was mixed correctly. Next, At 510 nm, the pink mixture's absorbance was measured using water as a reference. To produce a reliable calibration curve, several catechin solution mixtures were used. The findings were represented in mg of catechin equivalent (CE) for each gram of dry solids.

2.7. Syneresis analysis

Falcon tubes containing precisely 5 mL of sample were centrifuged at 500 rpm for 15–20 min at 4–5°. Within 1–2 min, the whey was separated. The volume of whey separated per 100 mL of yogurt was used to express the quantity of whey. The syneresis was evaluated and measured during 0th, 7th, 14th, and 21st.

2.8. Sensory evaluation

The sensory evaluation of yogurt samples used a 9-point hedonic scale for the research. A panel of several qualified judges with expertise in the study's topic evaluated sensory qualities. E.g., 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, and 9 = excellent.

2.9. Statistical analysis

The collected data were imported into SPSS for variance analysis (ANOVA). The significance level among the obtained mean values was determined using Duncan's Multiple Range (LSD) test.

TABLE 3 Effect of adding SPP and MPP on the chemical composition of Yogurt.

Parameters	Treatments								
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Average
SNF %	*8.98 ± 0.01[g]*	*9.66 ± 0.02[f]*	*9.99 ± 0.02[e]*	*10.11 ± 0.01[c]*	*10.30 ± 0.01[d]*	*11.00 ± 0.03[b]*	*11.18 ± 0.02[a]*	*10.18 ± 0.01[de]*	10.17 ± 0.60
TSS %	*12.88 ± 0.02[f]*	*13.45 ± 0.01[e]*	*13.51 ± 0.04[c]*	*13.71 ± 0.01[b]*	*13.55 ± 0.01[cd]*	*13.78 ± 0.17[b]*	*13.98 ± 0.01[a]*	*14.12 ± 0.03[e]*	13.55 ± 0.35
Protein %	*15.12 ± 0.02[a]*	*14.98 ± 0.02[b]*	*14.24 ± 0.06[e]*	*14.22 ± 0.08[g]*	*14.25 ± 0.02[c]*	*15.00 ± 0.03[f]*	*13.89 ± 0.04[h]*	*14.76 ± 0.06[d]*	14.55 ± 0.67
Fat %	*4.11 ± 0.02[a]*	*4.01 ± 0.02[b]*	*4.00 ± 0.01[c]*	*3.98 ± 0.04[d]*	*3.99 ± 0.06[d]*	*3.87 ± 0.01[e]*	*3.81 ± 0.02[f]*	*3.71 ± 0.04[c]*	3.93 ± 0.38
pH	*4.34 ± 0.05[d]*	*4.48 ± 0.03[c]*	*4.54 ± 0.04[b]*	*5.55 ± 0.01[a]*	*4.51 ± 0.03[e]*	*4.32 ± 0.04[e]*	*4.11 ± 0.06[f]*	*4.34 ± 0.04[e]*	4.52 ± 0.34
Ash %	*1.75 ± 0.03[g]*	*1.85 ± 0.01[f]*	*1.91 ± 0.03[d]*	*2.12 ± 0.02[b]*	*1.99 ± 0.01[e]*	*2.10 ± 0.04[c]*	*2.22 ± 0.03[a]*	*1.87 ± 0.00[ef]*	1.97 ± 0.16

Values in mean column are given in (Mean ± SD).
The Italic letter preset on each average value shows significant level. Means that do not share a letter in a column are significantly different at level ($p < 0.05$).
T0 = 0% Control.
T1 = 3% SPP.
T2 = 6% SPP.
T3 = 9% SPP.
T4 = 3% MPP.
T5 = 6% MPP.
T6 = 9% MPP.
T7 = 2.5 + 2.5% SPP + MPP.

3. Results

3.1. Compositional analysis

The average protein content of yogurt was (14.55 ± 0.67)/100 g, while 15.12 g was the highest we found in T0 with 0% dried MPP and SPP. The T6 sample with 9% freeze-dried MPP had the lowest protein content (13.89)/100 g. The fat analysis reveals that the average amount of fat in 100 g of yogurt was (3.93 ± 0.38) g, with the maximum amount (4.11/100 g) observed in T0 sample with 0% freeze-dried SPP and MPP and the lowest value (3.71/100 g) found in T7 sample with 9% MPP. Yogurt had total soluble solids in an average amount of (13.55 ± 0.35) w/w, found by Brix determination, the T6 (13.98% w/w%) sample containing 9% freeze-dried MPP had the largest quantity. While the T0 sample had (12.88)w/w% TSS content which is lowest at 0% freeze-dried powder. The solid and non-fat content of yogurt had an average value (10.17 ± 60)w/w% and the most significant amount (11.18)w/w% was found in T6 with 0% dried SPP and MPP, whereas the least (8.98 ± 01)w/w% SNF content was calculated in T0 sample with 0% freeze-dried MPP and SPP. To determine the acidity of milk pH analysis was performed and give $H+$ content or uptake in milk. The relationship between acidity and pH levels had simply a rough estimate. The pH analysis demonstrates that yogurt has pH value on average (4.52 ± 04). In contrast, the largest value was identified in T3 (5.55) sample with 9% freeze-dried SPP and the lowest pH value was analyzed in T6 (4.11 ± 01) sample with 9% MPP.

The ash level of the functional yogurt produced was evaluated where the ash concentration of furnace oil after it has been completely burned reveals the non-combustible portion that left. This was conducted to assess the quantity and makeup of minerals (Inorganic mass) in food. According the analysis of the ash, the average ash content was (1.97 ± 0.16)/100 g of yogurt. T6 had the highest value of ash (2.22 ± 01)/100 g with 9% freeze-dried MPP and control (T0) had the minimum value (1.75 ± 01)/100 g with 0% dried powder. The chemical composition of yogurt are depicted in Table 3.

The average chemical composition of SPP and MPP has been displayed in Table 4, showing that MPP samples had higher levels of ash (2.45), carbohydrate (57.14%), moisture (7.43)/100 g, and fiber (23.43), in comparison to SPP samples. SPP samples were discovered to have the maximum fat (1.12/100 g) contents and protein (8.53/100 g).

Except for protein and fat levels, the SNF, ash, Brix, and pH values of various treatments tended to rise when MPP or SPP concentrations increased (Figure 1). Furthermore, putting freeze-dried MPP components to the created functional yogurt elevated levels much higher than SPP integrated treatments ($p < 0.05$).

3.2. SPP and MPP bioactive potency

In Table 5 the bioactive potential of MPP and SPP have been exhibited which reveals that MPP samples had considerably greater TFC (26.151.0) CE mg/g levels and TPC (221.771.79)

TABLE 4 Proximate chemical analysis.

Product	Protein (g/100 g)	Fat (g/100 g)	Carbohydrates (%)	Moisture (g/100 g)	Fiber (g/100 g)	Ash (g/100 g)
SPP	8.53	1.12	53.13	6.68	13.55	1.23
MPP	5.45	0.06	57.14	7.43	23.43	2.45

SPP, soybean peel powder; MPP, mung bean peel powder.

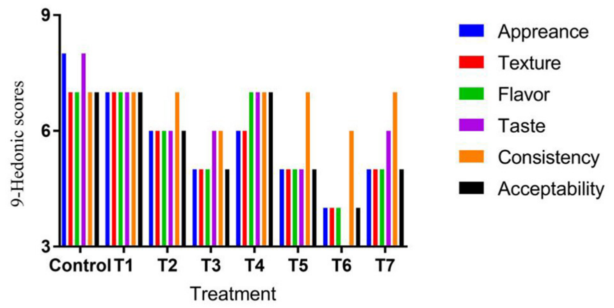

FIGURE 1
SPP and MPP effects on the rheological properties of functional yogurt.

TABLE 5 Bioactive profile of SPP and MPP.

Attributes	SPP	MPP
TPC (GAE mg^{-g})	52.36 ± 1.22^{b}	221.77 ± 1.79^{a}
TFC (CE mg^{-g})	8.40 ± 0.13^{b}	26.15 ± 1.00^{a}

Means that do not share a letter in a column respective to their factor are significantly different at level ($p < 0.05$). Values in mean column are given in (Mean ± SD).

TABLE 6 Effect of adding SPP and MPP on the bioactive profile of Yogurt.

Treatments	Parameters		
	Antioxidant activity (%)	TFC (CE mg/g)	TPC (mg GAE/g)
T_0	58.44 ± 0.04^{h}	1.23 ± 0.01^{g}	3.11 ± 0.01^{h}
T_1	60.21 ± 0.02^{g}	1.24 ± 0.03^{f}	3.40 ± 0.04^{g}
T_2	62.98 ± 0.01^{f}	1.35 ± 0.02^{e}	3.53 ± 0.03^{e}
T_3	64.11 ± 0.02^{e}	1.39 ± 0.04^{d}	4.32 ± 0.04^{c}
T_4	79.46 ± 0.01^{c}	1.52 ± 0.00^{c}	3.99 ± 0.02^{d}
T_5	82.11 ± 0.02^{b}	1.62 ± 0.03^{b}	4.11 ± 0.03^{b}
T_6	82.99 ± 0.02^{a}	1.74 ± 0.05^{a}	4.65 ± 0.05^{a}
T_7	68.23 ± 0.03^{d}	1.32 ± 0.04^{e}	3.33 ± 0.02^{f}
Average	69.81 ± 9.44	1.42 ± 0.16	3.80 ± 0.37

Values in mean column are given in (Mean ± SD).
Means that do not share a letter in a column are significantly different at level ($p < 0.05$).
T0 = 0% control.
T1 = 3% SPP.
T2 = 6% SPP.
T3 = 9% SPP.
T4 = 3% MPP.
T5 = 6% MPP.
T6 = 9% MPP.
T7 = 2.5 + 2.5% SPP + MPP.

mg GAE/g content. On the other hand the SPP samples had lowest TFC (8.400.13) CE mg/g and TPC (52.361.22) mg GAE/g.

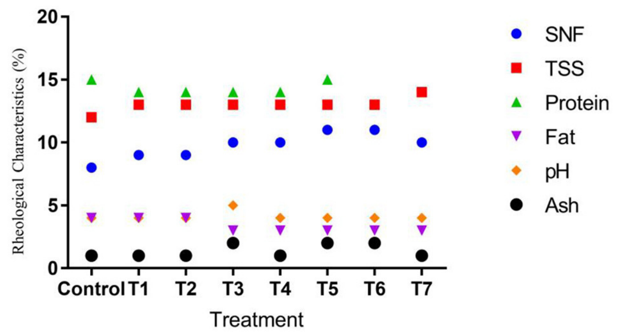

FIGURE 2
SPP and MPP effect on biological properties of functional yogurt.

3.3. Functional yogurt's bioactive profile

The descriptive analysis reveals average (3.80 ± 0.37)mg GAE/g concentrations in Table 6 for the TPC study. However, in T6 the highest concentration (4.65) mg GAE/g was found with 9% freeze-dried MPP, while in T0 the lowest TPC (3.11) mg GAE/g was found with 0% freeze-dried powder. The results of the TFC examination showed average (1.42 ± 0.16) CE mg/g values. The maximum TFC value (1.74) CE mg/g with 9% freeze-dried MPP was examined in T6 sample, whereas in T0 (Control) with 0% freeze-dried powder, the minimum TFC level was examined (1.23) CE mg/g. According to tests for antioxidant activity, an average of (69.81 ± 9.44)% was found. Furthermore, the highest DPPH inhibition or antioxidant activity with 9% freeze-dried MPP was detected in T6 (82.99). In comparison, the lowest DPPH inhibition or antioxidant activity sample with 0% freeze-dried powder was detected in T0 (Control) (58.44)%, as presented in Figure 2.

3.4. Syneresis in functional yogurt

Using a technique described in the materials and methods, prepared functional yogurt samples made up of (T0, T1, T2, T3, T4, T5, T6, and T7) were examined for their syneresis at various time intervals (day 0, day 7th, day 14th, and day 21st). Descriptive analysis was carried out, although the highest concentration was seen and measured in T0 (1.92 ± 0.00)/100 g during the 21st day of storage. The lowest Syneresis concentration was seen and calculated in T6 (1.42 ± 0.00)/100 g at the first day of storage. Each treatment received three replications, as indicated in Table 7.

3.5. Sensory evaluation

Table 8 shows the results of the created functional yogurt with MPP and SPP at various levels. In terms of descriptive analysis, the highest scores for Flavor (7.41), Consistency (7.10), Appearance (8.4), Taste (8.61), and Texture (7.81), with no *SPP and *MPP concentrations were discovered in T0 (Control) sample. In contrast, the lowest scores for Flavor (3.99), Appearance (4.56), Texture (5.00), and Taste (3.33), with 9% MPP were explored in T6 sample. Overall acceptability ratings were found to be much higher than those of other treatments examined. In this investigation, sensory qualities appeared to diminish as frozen powder concentrations increased. However, by incorporating SPP into yogurt, sensorial ratings did not drop substantially compared to treatments containing MPP. To facilitate comparison, estimated marginal means were calculated and textured for sensory evaluation of samples (Figure 3).

4. Discussion

Food sector creates a lot of waste, such as soybean and mung bean peel (Freitas et al., 2021). Freeze-drying method is used to eliminate moisture from soybean and mung bean peels. This study have been conducted to use peels of two different beans to prepare functional yogurt. Employing freeze-drying, the moisture level of MPP was found to be between 8.3 and 7.9 g per 100 g. When freeze-drying was used, the moisture content was 7.88/100 g. Compared to SPP, MPP samples had the highest fiber 35.19 and ash 3.53/100 g. These findings were noticed as 34.05–39.13/100 g for protein and 3.30–3.41/100 g for ash in MPP samples. TFC (26.15)mg GAE/g and TPC (221.71)mg GAE/g were the greatest in freeze-dried MPP samples, with similar results obtained in methanolic extracts. Thermal treatments are adverse to polyphenols in the food chain, as anticipated by Fischer et al. (2011) and Li et al. (2022).

A study Wojdyło et al. (2007) reported that polyphenolic contents tend to fall dramatically. According to Morais et al. (2018), this could be because most phenolic substances are bound to cell materials. Following drying activities will probably release the related biologically active ingredients from the food, making it more bio-accessible during the extraction technique. Freeze-drying is one of the most efficient and effective dehydration techniques, with enhance shelf life (Fischer et al., 2011; Ahmad et al., 2021), while Jovanović et al. (2020) say that freeze-drying is the greatest approach for preserving phytonutrients and fruit and vegetable powders' bioactivity (Babar et al., 2021). The results of this study suggest that freeze-drying might be employed to enhance the recovery of bioactive substances such as TFC and TPC from mung bean and soybean (Khonchaisri et al., 2022).

The pH level raised regarding the inclusion of SPP, but the pH level decreased dramatically at increasing concentrations of MPP. Similar behavior was reported during the creation of peanut milk

TABLE 7 Descriptive analysis for syneresis in different treatments.

Time intervals	Attribute-syneresis (g/100 g)								
	Treatments								
	Control	T1	T2	T3	T4	T5	T6	T7	Means
Day 0	1.75 ± 0.00	1.71 ± 0.00	1.68 ± 0.00	1.47 ± 0.00	1.70 ± 0.00	1.65 ± 0.00	1.42 ± 0.00	1.69 ± 0.00	1.64 ± 0.12^{D}
Day 7th	1.83 ± 0.00	1.74 ± 0.00	1.71 ± 0.00	1.49 ± 0.00	1.72 ± 0.00	1.69 ± 0.00	1.45 ± 0.00	1.71 ± 0.00	1.67 ± 0.13^{C}
Day 14th	1.88 ± 0.01	1.77 ± 0.01	1.73 ± 0.00	1.54 ± 0.00	1.75 ± 0.00	1.71 ± 0.00	1.49 ± 0.00	1.76 ± 0.00	1.71 ± 0.12^{B}
Day 21st	1.92 ± 0.00	1.91 ± 0.00	1.76 ± 0.00	1.58 ± 0.00	1.78 ± 0.00	1.74 ± 0.00	1.52 ± 0.00	1.79 ± 0.00	1.74 ± 0.12^{A}
Means	1.85 ± 0.07^{A}	1.76 ± 0.04^{B}	1.72 ± 0.03^{CD}	1.52 ± 0.05^{E}	1.74 ± 0.03^{C}	1.70 ± 0.03^{D}	1.47 ± 0.04^{F}	1.74 ± 0.04^{BC}	

Values are given in (Mean ± SD).
Means that do not share a letter are significantly different at level ($p < 0.05$).
T0 = 0%.
T1 = 3% SPP.
T2 = 6% SPP.
T3 = 9% SPP.
T4 = 3% MPP.
T5 = 6% MPP.
T6 = 9% MPP.
T7 = 2.5 + 2.5% SPP + MPP.

TABLE 8 Effect of adding SPP and MPP on the sensory profile of Yogurt.

Treatments	Sensory attributes					
	Appearance	Consistency	Texture	Flavor	Taste	Overall acceptability
T_0 (Control)	8.40[a]	7.30[a]	7.81[a]	7.41[a]	8.61[a]	7.85[a]
T_1	7.45[b]	7.10[c]	7.45[b]	3.60[b]	7.31[b]	7.46[b]
T_2	6.00[e]	6.98[e]	6.10[d]	4.99[g]	6.58[d]	6.50[d]
T_3	5.50[g]	6.66[h]	5.80[g]	5.11[g]	5.98[f]	5.61[g]
T_4	6.60[c]	7.10[b]	7.10[c]	6.44[c]	6.78[c]	7.22[c]
T_5	6.00[f]	6.99[d]	5.64[e]	5.55[e]	5.37[g]	5.99[f]
T_6	4.56[h]	6.77[g]	5.00[h]	3.99[h]	3.33[h]	4.99[h]
T_7	6.20[d]	7.00[f]	5.41[f]	5.78[f]	5.59[e]	6.10[e]

Values are given in (Mean ± SD).
Means that do not share a letter in a row are significantly different at level ($p < 0.05$).
T0 = 0% control.
T1 = 3% SPP.
T2 = 6% SPP.
T3 = 9% SPP.
T4 = 3% MPP.
T5 = 6% MPP.
T6 = 9% MPP.
T7 = 2.5 + 2.5% SPP + MPP.

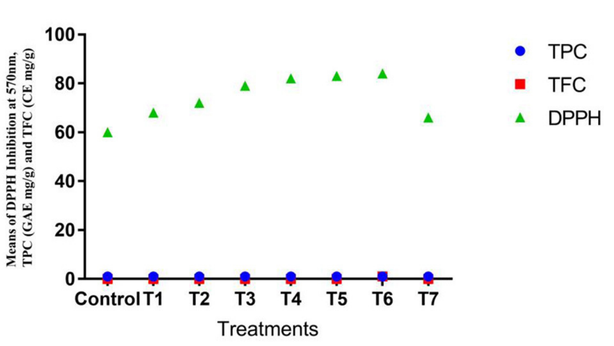

FIGURE 3
Sensory evaluation estimated marginal means for functional yogurt samples.

fermented curd (O'Rell and Chandan, 2013). Thus, in the context of soybean, the pH likely to rise, resulting in a decrease in the acidity of yogurt, perhaps connected to the diluting effect. Ash contents, Total soluble solid, and solid not fat were dramatically enhanced after adding SPP and MPP in ascending order. Similar rise found in TSS and SNF quantities when fiber-enriched yogurt had developed (Nagaoka, 2019). The addition of SPP and MPP concentration order was found to significantly affect protein and fat levels; The Food and Drug Administration's (FDA) criteria for drinkable yogurt stipulate that it must include, 0.5% non-fat, >8.25% milk (SNF), 2% low fat, and >3.25% yogurt before any further ingredients are added. Yogurt that has been fiber-enriched and contains SPP and MPP falls under the category of low-fat yogurt.

The 21st day of storing, the highest syneresis level was detected and measured in T0 (1.79 ± 0.01)/100 g, T6 seemed to have the lowest syneresis value on day one of storage (1.40 ± 0.01)/100 g. The combination of SPP and MPP decreased syneresis concentrations. Meanwhile, treatments with MPP (1.42 ± 0.01)/100 g showed that treatments containing SPP (1.71 ± 0.01)/100 g had reduced syneresis on 21st day of storage.

T6 with 9% MPP had the maximum TFC (1.74) CE/g and TPC (4.65) GAE/g levels. TPC and TFC levels were considerably higher in the present study when SPP and MPP concentrations in yogurt increased. Meanwhile, the addition of SPP and MPP resulted in a significant increase in bioactive substances compared to adding only SPP. A comparable increased in TFC and TPC chemicals has been reported during the creation of probiotic yogurt enriched with SPP (Yadav et al., 2010). TPC and TFC were shown to be significantly increased by supplementing mung bean peel extracts during the formation of stirred yogurt, with values ranging from 1.11–2.18 mg CE/g to 3.39–5.97 mg GAE/g respectively (Fischer et al., 2011). It was established that the amounts of TPC and TFC in various generated treatments were strongly associated with the levels of MPP and SPP. Additionally, by using freeze-dried MPP, the concentrations of TPC in functional yogurt developed were much greater than freeze-dried SPP. These results were found in engagement with the studies led by Issar et al. (2017) and Wang et al. (2020).

Antioxidant scavenging activity is based on how many phenolic and flavonoid compounds are present in a specific diet. As the previous pattern continued, considerable antioxidant activity was seen as SPP and MPP concentrations increased gradually. SPP and MPP concentrations were shown to be closely linked to the antioxidant activity of various generated treatments. By including freeze-dried MPP, antioxidant activity in generated functional yogurt increased much more than freeze-dried SPP (Issar et al., 2017; Wang et al., 2020). Earlier, findings (Yadav et al., 2010; O'Rell and Chandan, 2013; Wang et al., 2020) highlighted that the addition of SPP and MPP amplifies the antioxidant action of yogurt.

5. Conclusion

In this study, we made functional yogurt with SPP and MPP and tested it for bioactive profiles such TFC, TPC, and DPPH inhibition, commonly recognized as antioxidant activity. Soybean and mung bean peel were freeze-dried first, then added to yogurt. Except for protein and fat content, which were much greater in SPP samples, Chemical composition and bioactive study clearly show that MPP outperforms SPP. Moreover, when MPP or SPP were combined to functional yogurt, the rheological properties were observed to be moderately improved in contrast to the control sample. Furthermore, bioactive characterization of functional yogurt was performed, demonstrating considerably better effects in MPP-containing therapies. The sensory qualities of beneficial yogurt samples were also evaluated, which explain that by introducing greater levels of SPP or either MPP in both circumstances, the sensory profile looks to be weakening; this decrease was shown to be more significant in MPP (T4, T5, and T6) added treatments compared to SPP (T1, T2, and T3) introduced ones. In this study, the SPP and MPP enhanced yogurt was revealed to be the maximum suited in relation to taste, appearance, body/consistency, and overall acceptability, showing that soybean and mung bean peel may be used in the food sector as a basis of bioactive components in yogurt after freeze-drying.

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

MS and AT contributed to conception, design of the study, and performed the statistical analysis. MA, AK, LB, and SH contributed to resources and validation. MS wrote the first draft of the manuscript. MS, AT, LS, MA, SH, and LB contributed to writing, review, and editing the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

LB is employed by Belrosakva Limited Liability Company, Minsk, Belarus.

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

Publisher's note

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Tea polyphenols: extraction techniques and its potency as a nutraceutical

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Usually, polyphenols help address numerous health issues caused by oxidative stress. Tea is a popular beverage (rich in polyphenols) with abundant health promoting and disease prevention with great health-promoting and disease-prevention attributes, originating from the delicate, dried leaves of the *Camellia sinensis* plant. Tea has been proven to have health-boosting impacts like anti-inflammatory, anti-cancerous, anti-diabetic, and aids in weight loss. Cognitive impairment, also known as cognitive decline caused by aging or other neurological disorders, has become an emerging health concern. Tea polyphenols, especially phenolic acids, havegained enormous attention due to their link to improved cognitive function by preventing cognitive decline. This review summarizes recent studies on the health benefits of polyphenols in tea. Additionally, effective traditional and modern techniques to extract polyphenols and their effects on various diseases have been described.

KEYWORDS

tea-polyphenols, extraction techniques, antioxidants, nutraceuticals, diseases

1. Introduction

Oxidative stress negatively affects human metabolism, which has a large global influence on direct and indirect health disparities. Among the many treatment modalities, polyphenol-based dietary therapies stand out for their capacity to restore the equilibrium between antioxidants and free radicals, hence lowering the risk of oxidative stress (Manzoor et al., 2022). The four primary polyphenols are phenolic acids, lignans, stilbenes, and flavonoids. Many *in-vivo* and *in-vitro* investigations have been conducted to evaluate the health impacts of polyphenols. They play a crucial part in defending the body from external stimuli and getting rid of reactive oxygen species (ROS) are directly linked to numerous diseases. Polyphenols, possessing health-boosting potential, can be found in tea, cocoa, fruits, and vegetables (Rana et al., 2022). Polyphenols are employed in medicine, cosmetics, nutraceuticals, and food because of their antioxidant, anti-inflammatory, anti-apoptotic, anti-carcinogenic, and antibacterial characteristics (Rajha et al., 2022; Noreen et al., 2023). Naturally, polyphenolic compounds are present abundantly in tea and coffee and have antioxidant and neurotoxic properties (Ali et al., 2021a). Additionally, polyphenols have been found to prevent and improve COVID-19 infection. The brain has weak antioxidant activity compared to other body organs, hence containing higher levels of ROS, making it more vulnerable to neurodegenerative diseases, including Alzheimer's and Parkinson's (Ali et al., 2022a; Liczbiński and Bukowska, 2022).

Around the globe, tea is known to be the primary source of flavonoids, accounting for more than half of total consumption (He et al., 2021). Various studies established that tea and its flavonoids might aid cardiovascular health (Adhav and Deore, 2022). Tea consumption has been linked to oxidation, anti-inflammation, cancer prevention (Li et al., 2022), cardiovascular preventative medicine, and other health advantages (Fan et al., 2021; Ali et al., 2022b).

The tea plant leaves, native to China and other Asian countries, are used to make tea (Pan et al., 2022; Rasool et al., 2023). Tea polyphenols (TPPs), caffeine, and other beneficial chemicals are found in almost all forms of tea. Tea is derived from the Southeast Asian plant Camellia. Flavonoids seem to be the most common flavonoid subclass, which may be found in pharmaceutical teabags (Fantoukh et al., 2022; Iqra et al., 2023). Flavan-3-ols and their oligomers, flavonols and their glycosides, phenolic acids and hydrolysable tannins, theaflavins, and thearubigins are only a few of the several types of polyphenols found in tea (Figure 1).

According to previous studies health boosting potential of tea is mainly due to tea polyphenols (Yan et al., 2020; Riaz et al., 2022). TPPs are well-known for their oxidative, anti-cancer, and other therapeutic characteristics (Mukhtar and Ahmad, 2000; Xing et al., 2019), but studies have shown that substantial amounts of tea are required to reap these advantages. Polyphenols are naturally occurring compounds in fruits and vegetables with significant antioxidant properties (Luo et al., 2021). Polyphenols prevent the oxidation of cells hence preventing neurological disorders. Tannin has been shown to protect neuronal cells in a Parkinson's disease model (Ali et al., 2021b; Xu et al., 2023). For decades, many plants have been known for their therapeutic and nutritional properties and are used in several traditional medicinal compositions (Satti et al., 2019). In the average human diet, fruits and veggies are the primary source of bioactive components. Bucciantini explored several polyphenol sources and their therapeutic effects on various human chronic disorders (Bucciantini et al., 2021). Previous studies explored the therapeutic potential of crucial phenolic acids like rutin, carvacrol, and green tea catechins, highlighting the medicinal qualities of polyphenols against Amyotrophic lateral sclerosis (ALS) and Developmental disorders (Novak et al., 2021). Tea polyphenols, active phytochemicals and vitamin C that reduce inflammation, are also found in black tea. Tea polyphenols have strong antioxidant capabilities due to their capacity to absorb electrons and metals (Nobahar et al., 2021; Aziz et al., 2022). This article discusses previous work on the types of polyphenols found in tea, conventional and advanced TPP (tea polyphenols) extraction methods, and their health boosting mechanism against various disorders.

Abbreviations: ALS, amyotrophic lateral sclerosis; ATP, adenosine triphosphate; BMI, body mass index; CTC, cut/crush, tear, and curl; ChCl, chromium chloride; ECG, epicatechin-3-gallate; EGCG, epigallocatechin-3-gallate; EG, ethylene glycol; DES, deep eutectic solvent; GTP, green tea polyphenol; HDL, high density lipoprotein; HBD, hydrogen bond donors; HPE, high-pressure extraction; LDL, low density lipoprotein; MAE, microwave-assisted extraction; NO, nitric oxide; PHWE, pressurized hot water extraction; RSV, resveratrol; ROS, reactive oxygen species; SFE, supercritical fluid extraction; Tf3, theaflavin-3, 3'-digallate; TPPs, tea polyphenols; UAE, ultrasound-assisted extraction.

2. Different forms of tea

It is prepared from immature stems of the *Camellia sinensis* species (Rana et al., 2016; Engelhardt, 2020). Due to geographical variation, different teas express varied colors, flavors, and scents (Wang Z. et al., 2022). Freshly cut delicate tea stem is heated before rolling and dried to prevent lipid oxidation by lipases (phenolic acids oxidase and peroxidases) by implementing various techniques and mechanisms (Wang et al., 2021; Samanta, 2022). CTC black tea (cut/crush, tear, and curl) and traditional black tea are the two types of black tea. CTC black tea is produced using CTC machinery that breaks the stalk into little pieces before crushing and bending the tea granules after drying them (Deka et al., 2021; Ahmed et al., 2022).

Wide tea varieties have originated in China (Liu et al., 2019; Zhang et al., 2020). As mentioned above that, CTC black tea is made by deactivating all lipases (enzymes) and falls somewhere between green and black tea (Li T. et al., 2021).

2.1. Types of teas

Various countries produce diverse types of tea. For instance, China occupies a first place for the country with the most tea plants; additionally, herbal tea exports make around half of Chinese total exports worldwide. Black tea exports accounts for 30% of the total, while other teas account for 20% (Khalid et al., 2022b; Xu et al., 2022). Black tea is typically named by its cultivation area. Various regions are frequently recognized for manufacturing teas with distinctive flavors (Chen et al., 2020).

2.1.1. Black teas

Previous literature has shown several kinds of tea; Lapsang Souchong is a region in Fujian where black tea is cultivated on Mount Wuyi. It's indeed a blend of several black teas, mainly consisting of Assam teas and other black teas. Bengal tea has a flowery, fruity flavor. In India, Dooars is the most famous region for cultivating black tea, upto 32% from West Bengal state. This region is known for Orthodox tea, black teas and CTC (Malakar et al., 2022). Darjeeling tea with a distinct muscatel flavor comes from West Bengal and India. It is known as "champagne of teas" (Lama, 2022; Wang L. et al., 2022). An inexpensive Vietnamese tea with a heavier texture and darker brew than Nepal or Darjeeling teas. To form a beverage, black tea is combined with other plants. Earl Gray is a bergamot-flavored black tea. Tea usually drink with English breakfast is thick, rich, and full of sugar and milk (Rezaeinia et al., 2020; Yun et al., 2021; DeBernardi and Ma, 2022).

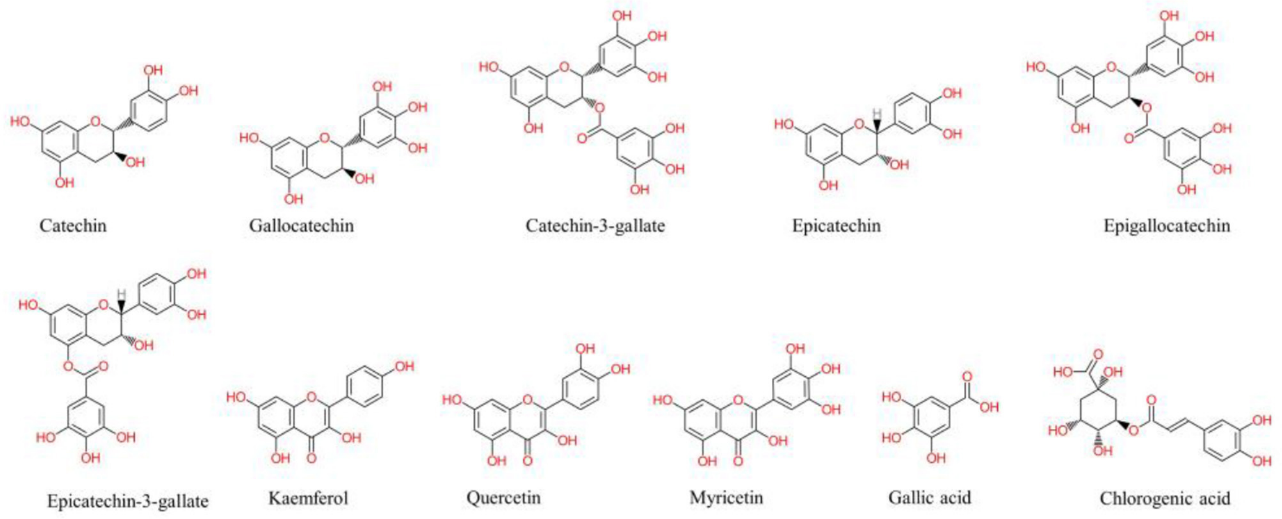

FIGURE 1
Major tea polyphenols and their chemical structures.

2.1.2. The effect of polyphenols in black tea on digestion, assimilation, and consumption

According to recent studies, tannins found in all type of teas but specifically in black tea (which contains more than other teas), aids in digestion by soothing the gastric tract and preventing GI track syndromes (Al-Mahdi et al., 2020). In a study conducted on 60 students between age of 18 to 23 years, mouth rinsing with green and black teas showed a significant increase in saliva pH and its flow rate. This increase in pH and flow rate of saliva improved the digestion of food and maintained oral health by inhibiting microbial infections (Shetty et al., 2020).

Protease coating the surface of lipid rafts is required for the inter-facial process of fat stomach acid. Droplet formation produces an exterior for the lysis buffer adsorbent surface that alters lysozyme mooring and thus influences the hydrolytic cost. In a functional prototype replicating tiny small intestine settings, during a thickener of 0.10 mg dosage of black leaf extracts, the initial particle size altered from 1.4 to 25.9 m (Wojtunik-Kulesza et al., 2020; Ziółkiewicz et al., 2023). The use of black tea extract delayed droplet dispersion and reduced surface area. Fat gastric acid's interface phase necessitates protease's presence on lipid rafts' interface. Droplet production creates a surface for adsorbed species from lysis buffer, influencing the lipase anchoring and hence the price of hydrolytic breakdown, in a functional prototype replicating microscopic small intestine conditions. The preparatory pressure loss of 0.10 mg of black tea during emulsifying agents usually ranges from 1.4 to 25.9 m. A black tea core is used to delay particulate dissolution and reduce area (Kan et al., 2020; Vivarelli et al., 2023). On highly fed obese rats, high-purity project producers were tested for pro and lipid membrane action (Jin et al., 2013; Takemoto et al., 2016). Moreover, other research has revealed that drinking black tea while maintaining a healthy diet can help lose weight (Lin et al., 2021). Black tea polyphenolic compounds can potentially change the lipid content of stools, thereby reducing weight gain caused by a high fat diet (Wu et al., 2016).

2.1.3. Green Tea

Chinese green teas come in various textures, including striped "Meecha," spherical "Gunpowder," and flat "Longing." Oolong Tea: This tea's aroma dates back to the 18th century. Tea with varying degrees of fermentation is known as "Pouchong," which is mildly fermented, or "Tie-Qian Yin," which is severely handled. They were famous among men and women due to their low-calorie count and ability to aid in weight loss. The color of such tresses distinguishes white and yellow tea on the ganja and the leafy beneath the earth (Cao et al., 2022; Bhandari et al., 2023).

2.1.4. Tea and tea-based functional beverages

Recently tea has been utilized to develop various kinds of functional beverages. Due to their proven health-boosting potential, such as reduction in weight, anti-oxidant attributes, refreshment purpose and alternative to supplements and energy drinks, tea-based functional beverages gained enormous recognition worldwide (Jayabalan and Waisundara, 2019). Tea-based functional drinks like Kombucha tea, are developed by fermenting tea and sugar by infusion of yeasts and bacteria turning into a "tea fungus" like form.

The fermentation process takes 2 weeks; green or black tea can be used as a base (Sinir et al., 2019). Bioactive constituents, organic acids and tea polyphenols are the basic active compounds that make kombucha a functional beverage. Although consuming functional beverages has been linked to improved immunity, better nervous system function and GI track immunity, more research should be conducted on fermentation procedures' safety and hygiene levels. It is recommended to analyze any toxicity levels in functional beverages due to prolonged fermentation processes to ensure their safety in future (Dini, 2019).

2.1.5. Other by-products of tea

Tea is among the most prevalent drink, mostly as a standalone soft drink and as an element of other beers. Several studies have discussed tea and its constituents' possible medical advantages. White tea, dark tea, yellow tea, and matcha are currently among the most famous green tea in the world. Teas are divided into categories like oxygenation tanks teas, oolong, white, black teas, dark teas, and unfermented teas, such as herbal tea. Pekoe and Bright Red Pekoe are black teas. "Pek-ho" refers to gold Assam teas in Chinese and indicates "white hair." Peach Pekoe is usually produced from the top leaf of trees in Pakistan or Sri Lanka. Pekoe tea is known in India, Indonesia, and Asia for having even narrower leaves than Peach Pekoe tea (Kłopotek and Dmowski, 2022). Green and black teas are popular in Asian nations, although black tea is common in Western countries (Gunec, 2023).

3. Extraction techniques for tea polyphenols

Extracting TPs has been done in a variety of ways. Maceration extraction is one of the conventional techniques for TP extraction (also known as solvent extraction). Innovative extraction techniques, for example, deep eutectic extraction, high-pressure, pressurized hot water, supercritical fluid, and procedures aided by microwave and ultrasound, have been known for some years now, as shown in Figure 2. The different extraction methods are described in more details in Table 1.

3.1. Conventional maceration extraction

3.1.1. Water extraction

For the TP extraction, hot and cold water was utilized. Prior studies examined the phenolic profiles of 30 different tea products using hot water extraction at 98°C, and the findings revealed that some dark and black teas may contain more gallic acid than other types of tea (including white, yellow, black, and dark tea). Green tea is said to have more catechins than other types of tea (Tang et al., 2019). Up to 5 min of infusion time, according to another study (Pérez-Burillo et al., 2018), had minimal effect on the yields of TPs from white tea. However, after that point, TP yields rose with infusion duration. Improved TP yields are associated with longer extraction durations; however, because TPs are thermally destroyed, overly hot water extraction must be avoided. For instance, the total quantity of flavonoids dropped when bagged green tea was extracted with hot water (80°C) for over 15 min (Dai et al., 2022). Cold-water extraction appears to be more efficient than hot-water extraction for the extraction of TPs. It claimed that one reason contributing to this behavior is the phenolic components' breakdown when exposed to heat. The infusion times for cold-water extraction are occasionally significantly longer than for hot-water extraction. The size of the tea particle depends on the mechanism of the extraction process (Jahanfar et al., 2021).

Tea samples and solvents have a large contact area because smaller particle diameters and greater specific areas are correlated. The yield of TPs increased by 14% compared to unbiased tea leaves, which weighed 61% more and had widths ranging from 0.15 to 0.74 mm. Overall, the extraction efficiency is greatly influenced by temperature, infusion time, and particle size. There are no safety concerns with the solvent employed in the water extraction. Both hot water extraction and cold water extraction have their pros and cons. For instance, the cold water extraction requires more processing time, while hot water extraction might cause TP deterioration (Rahaman et al., 2020; Manzoor et al., 2021, 2023; Lomartire and Gonçalves, 2022).

3.1.2. Organic solvent extraction

The TPs must be soluble in the organic solvents to be extracted properly. According to recent research on TP extraction in Australian tea shoots, methanol was a better solvent than water, ethyl acetate, and ethanol. Extended extract times for the first 8 h significantly enhanced TP yields. However, values changed after 8–18 h (Jiajia et al., 2021).

Black tea was previously processed to extract theaflavins and thearubigins using water, ethanol, and methanol. The results showed that ethanol was more effective in extracting these TPs (Imran et al., 2018). In this study, all of the solvents attained their maximal extraction after 60 min, and when the extraction time was extended, the recovery decreased, most likely due to heat degradation (Imran et al., 2018).

3.2. Novel extraction techniques

3.2.1. Ultrasound-assisted extraction

By creating cavitation bubbles, ultrasonic waves may alter the medium's pressure and temperature, upsetting the sample cells and accelerating the movement of bioactive substances (Gao et al., 2022). Ultrasound treatment is a popular alternative to evaluating certain bioactive components (Rahaman et al., 2021; Piasecka et al., 2022). Ultrasound-assisted extraction (UAE) has the power to increase TP yields. UAE might significantly shorten the time it takes to extract green tea without increasing the amount of unwanted caffeine that is eliminated (Kang et al., 2022).

Likewise, compared to hot water extraction, the UAE with ethanol substantially doubled the amount of EGCG in green tea extract (Ayyildiz et al., 2018). According to a prior study, sonication did not affect the antioxidant activity of green tea extracts between 45 and 55°C, as the temperature increased, the antioxidant properties dropped (Xu et al., 2019). When extracting polyphenols from branded black tea products, the ultrasonic sensor at a frequency of 26 kHz was more effective than the ultrasonic bath at a rate of 40 kHz while using less energy (Bakht et al., 2019; Ali et al., 2022c).

3.2.2. Microwave-assisted extraction (MAE)

It has been investigated how to extract phenolic compounds and essential oils using microwave-assisted extraction (MAE) of bioactive substances (Singh et al., 2022). The three stages of the MAE process include the diffusion of the solutes from the sample material into the solvent, the introduction of the solvent into the sample matrix, and the purification of the solutes from the active sites of the matrix (Hudiyanti et al., 2022). A significant amount

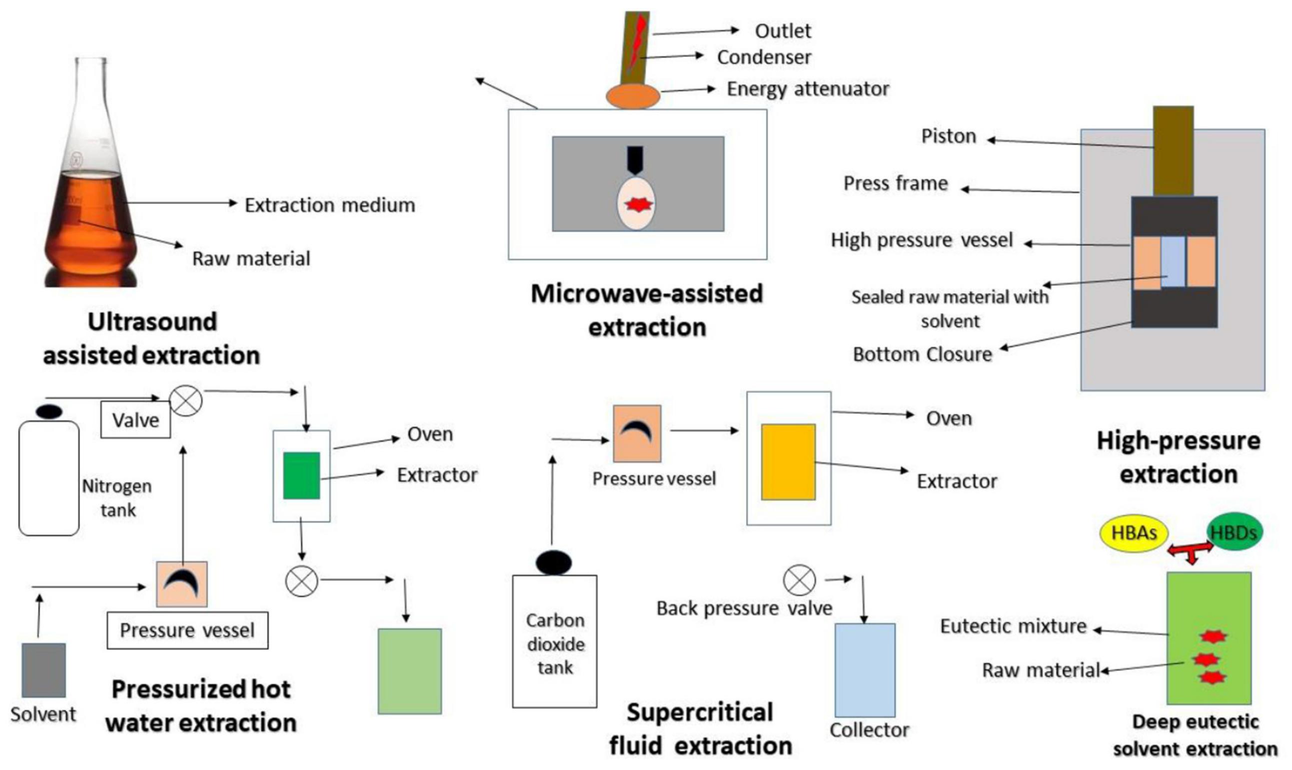

FIGURE 2
Innovative methods for TP removal. HBAs and HBDs are acronyms for hydrogen-bonding acceptors and distributors, respectively.

of polyphenols may be produced fast in microwaved tea products. Twenty hours of room temperature maceration, 90 min of UAE extraction, and 45 min of heat-reflux extraction were needed before MAE could extract 30% (w/w) polyphenols from green tea extracts (Dao et al., 2022). In a previous study, microwave time rather than microwave intensity, tea-to-water ratio, or the number of radiation exposures had the greatest effect on the number of green tea polyphenols (Li H. et al., 2021).

This also demonstrated that a microwave power of 600W, a tea-to-water ratio of 1:10, and a radiation duration of 3 min were the best radiation parameters. According to other research, microwaving the tea for 7.8 min and repeating the extraction three times were the ideal conditions for extracting polyphenols from green tea by MAE. The TPC of the extracts produced under these circumstances was higher than those produced by the UAE under optimal circumstances, coming up at about 96 GAE/g dry weight (Al-Hatim et al., 2022).

3.2.3. High-pressure extraction

High-pressure separation of bioactive components from plant materials has also been a popular non-thermal method (Khan et al., 2019; Manzoor et al., 2020). By allowing solvents to enter cells, high pressure speeds up mass transfer and the diffusion of active compounds (Ahmed et al., 2019; Tang et al., 2019). It seems that the duration of the pressure application has no impact on how much TP is removed. Prior research, for instance, discovered that tea leaf cells entered the solution immediately since the pressure-holding duration (between 1 and 10 min) had no discernible effect on the TPs' extraction yields (Lin et al., 2022). It's anticipated that the High-pressure extraction's (HPE) laboratory scale can be converted into an industrial scale.

3.2.4. Pressurized hot water extraction

As a green extraction method (an advanced technology applied to reduce the use of organic solvents to lower climatic and health-related issues and optimize the yield of required phenolic compounds via selective extrication methods), pressurized hot water extraction (PHWE) is used. Liquid water is used as the solvent in PHWE between 100°C (the boiling point of water at 0.1 MPa) and 374°C (the critical point of water at 22.1 MPa) (Plaza, 2019). The most important elements of the PHWE are temperature and flow rate. On the one hand, high temperatures can hasten polyphenol migration and improve sample wetting. Polyphenols, on the other hand, are delicate and easily harmed by high heat. Because TPs were exposed to higher temperatures over longer extraction times, TP degradation was further accelerated. For instance, the yields of (–)-epicatechin-3-gallate (ECG), epigallocatechin-3-gallate (), and catechin were inversely associated with temperature when the extraction time surpassed 28 min (He et al., 2018). Another crucial element for PHWE is the flow rate. High flow rates can speed up extraction and reduce the number of time analytes must be in the hot water, but if the flow rate is excessively high, it can contaminate extracts and further concentration procedures will be required (Kasapoglu et al., 2022).

TABLE 1 Different extraction methods, their impact on quality of TPs, advantages and disadvantages.

Technique	Pros	Cons	Impact on the quality	References
Water extraction	No safety concerns as no solvent is used	In CWE Prolonged infusion time is required HWE, deteriorates tea polyphenols particles	Purest form of TP without any traces of solvents Hot water extraction may damage TP's particles	Jahanfar et al., 2021
Organic solvent extraction	Wide range of solvents are available (water, methanol, ethanol, ethyl acetate, hexane) Easy to set up and low installment cost Purified product is obtained with SCF (CO_2 as solvent)	Prolonged exposure to solvents and high temperature High risk of degradation of compounds Huge amount of solvent is required Post process purification is a must	Selective compounds can be obtained by SCF extraction, without post process techniques More prone to epimerization than other techniques Solvent extracts (other than water) need to be purified	Imran et al., 2018
Ultrasound assisted extraction	Simple set up and easy to scale up No post processing required Low temperature application; can be used for heat-labile compounds	Long extraction time Lack of uniformity of wave distribution	May prone to epimerization if temperature is near 100°C	Bakht et al., 2019
Microwave assisted extraction	Maximum recovery Short exposure time (1–3 min) Less degradation of compounds (here polyphenols) Reduced solvent usage	Batch processing Post processing is required	Traces of solvent (if other than water is used) with compound Less degradation due to less exposure to heat as compared to other techniques	Al-Hatim et al., 2022
High pressure extraction	High pressure speeds up mass transfer and the diffusion of active compounds Non-thermal	–	No degradation due to less exposure to heat as compared to other techniques	Lin et al., 2022
Pressurized hot water extraction	Reduce the use of organic solvents Optimize yield	High pressure can deteriorate the TPs particles	High flow rate contaminates the extract	Kasapoglu et al., 2022
Supercritical fluid extraction	Increase the extraction rate by altering the fluid's temperature, pressure, or both	Expensive technique Small sample sizes	CO_2 traces in final extract	Ashfaq et al., 2019
Deep eutectic solvent extraction	Good thermal stability Simple biodegradation, accessibility, simplicity of manufacture	Environmental toxicity	Some traces of solvents may remain in final product	Cui et al., 2021

3.2.5. Supercritical fluid extraction

Supercritical fluid extraction (SFE) is an extraction technique with little thermal damage, high mass transfer rates, and low temperatures (Jha and Sit, 2022). Since the fluid's density affects solute solubility, SFE can increase the extraction rate by altering the fluid's temperature, pressure, or both. Carbon dioxide (CO_2) is the substance that is most frequently employed in supercritical extraction because of its low critical temperature (31.3°C) and pressure (7.39 MPa) (Al Jitan et al., 2018).

But since CO_2 is a gas at ambient temperature, it is simple to remove from the extracts. It is possible to extract EGCG effectively using supercritical CO_2. EGCG concentration was higher in green tea extract produced with SFE (77.23 mg/g) compared to acetonic extract (65.88 mg/g), which was produced with constant extraction temperature and duration (Ashfaq et al., 2019; Shehzad et al., 2021). Additionally, SFE has disadvantages, including a high price and potentially small sample sizes.

3.2.6. Deep eutectic solvents extraction

In an advanced alternative to conventional organic solvents known as deep eutectic solvents (DESs), the hydrogen bond donors compounds such as urea, lactic acid, citric acid, ethylene glycol, and choline chloride are mixed in a eutectic combination (Santana-Mayor et al., 2021). DESs have some benefits, including good thermal stability, simple biodegradation, accessibility, simplicity of manufacture, and low toxicity (Cunha and Fernandes, 2018). Using ChCl-based DESs, tea polyphenols were extracted depending on the characteristics above. The ability of DESs to extract TPs has been demonstrated. Compared to conventional solvents, DESs have a better extraction efficiency for extracting TPs from green tea (water and ethanol). Numerous HBDs (hydrogen bond donors), including ethylene glycol (EG), glycerol, glucose, oxalic acid, and citric acid, have their extraction efficacies tested. Compared to other solvents, ChCl/EG (1:2 molar ratio) has a much greater extraction efficiency (Cui et al., 2021). More research should be done to test if TPs can be extracted using DESs and other solvents.

4. Pharmacological activities of tea polyphenols constituents

Tea is a popular beverage worldwide because it boosts health potential (Figure 3). Tea drinking has been linked to preventing and postponing several metabolic illnesses. It lowers the risk of heart problems, stroke, and high triglycerides, as well as irritating prevention, allergy prevention, high triglycerides, and

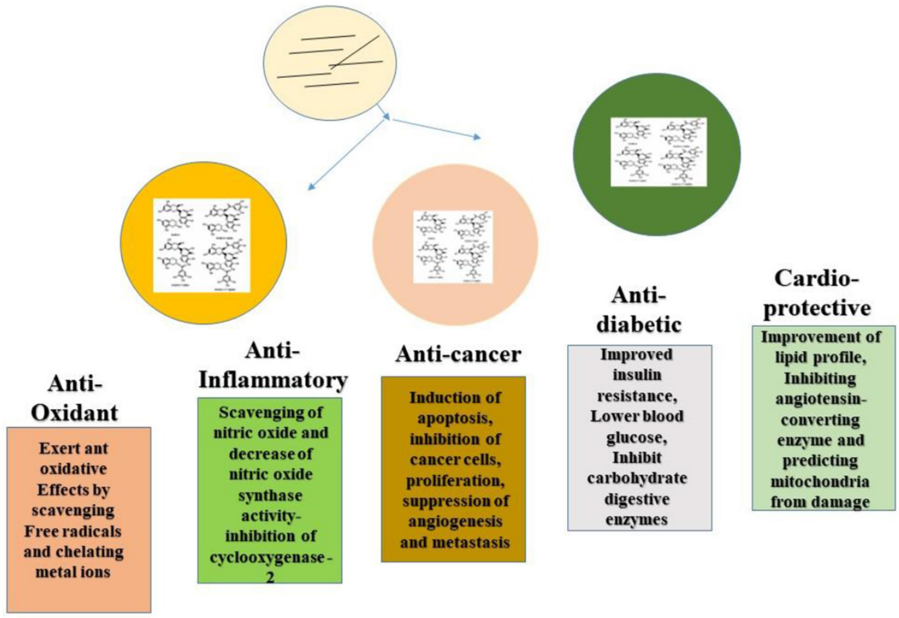

FIGURE 3
The physicochemical activities of major tea ingredients as an antioxidant, pro, anticancer, hypoglycemic, and neuroprotective effects.

irritating prevention of allergy, and other ailments (Shang et al., 2021). Antioxidant compounds are the most prevalent bioactive constituents found in tea, with the ability to scavenge free radicals and transfer those into stable molecules (Sena et al., 2020; Yan et al., 2020). In a recent large scale survey, green and black tea tannins prevent brain tumors by diminishing inhibiting various signaling molecules, including tumor necrosis factor, g-csf colonial power factor, and cox-2 (Bag et al., 2022). Tea, as well as its constituents, have been demonstrated in numerous trials to inhibit cancer in the skin, breast gland, lung, mouth, throat, esophagus, stomach, intestine, colon, liver, prostate, and pancreatic (Xu et al., 2020).

Phenolics in herbal tea have increased glycemic control, insulin sensitivity, and sensations in persons with type 2 diabetes. Tea phenolics are also thought to block carbohydrate digestion enzymes and decrease glucose transport within the GI tract (Sharma et al., 2019). Coffee catechins have been demonstrated to improve blood serum lipids by inhibiting major fat-producing enzymes (Ma et al., 2020). Tea and its flavonoids are gaining appeal as a reliable, powerful antioxidant that can help prevent heart disease (Yan et al., 2020). Several preclinical tests has emphasized tea catechins' therapeutic value in treating different diseases (Baldi et al., 2019; Babar et al., 2021). Tea consumption has been associated with a lower rate of various colon cancer (Zhao et al., 2022). Black and green tea preparations have been demonstrated to affect tumorigenesis translation, implying that they'd be utilized to treat pancreatic cancer. Herbal tea catechins may be utilized to treat brain diseases such as Parkinson's and Alzheimer's (Payne et al., 2022).

Alzheimer's infection is a global mental illness characterized by the formation of the amyloid clump from the -amyloid polypeptide (Lekwuwa et al., 2022; Ahmad et al., 2023). Both black and green polyphenol has antioxidant properties that aid in decreasing blood pressure (Ratnani and Malik, 2022). Flavanols, including flavonoids, exert diverse bioactive impacts, notably tooth decay prevention and anti-carcinogenic properties in the mouth (Xiao, 2022). Tea has been found in trials to enhance bone mass in women aged 65–76 (Biver et al., 2023). Flavonoids have the potential to suppress carcinogenesis via p53-mediated downregulation. Phenolic chemicals may interact with the epithelial lining of the gastrointestinal mucosa (Shehata et al., 2022).

5. Functional and nutraceutical aspects of tea

The predominant phytochemicals of beverage tea are catechins, theaflavins, and thearubigins (Manzoor et al., 2017; Datta et al., 2022). Several scientific studies established tea linkage to various health boosting potentials (Sharma and Rao, 2009; Engelhardt, 2020; Maqbool et al., 2022). Previous studies have discussed tea's disease control and prevention aspects against numerous pathological disorders such as obesity, insulin resistance, hypertension, cholesterol, and cancer (Anwar et al., 2022; Niewiadomska et al., 2022; Rana et al., 2022; Basu et al., 2023). Over the past decades, tea has been consumed globally for nutraceutical and. Due to its numerous health-boosting potential, specifically its free radical scavenging attributes, that suppresses the onset of degenerative ailments, infertility complications, a variety of malignancies, osteoporosis and insulin resistance (Nelson, 2022).

TABLE 2 Tea polyphenols have therapeutic properties.

Activities	Functions	References
Tea polyphenols		
Antioxidants	Aid in the preservation of cells and tissues from superoxide anion oxidative stress	Mao et al., 2017; Yan et al., 2020
Anti-inflammatory activity	Tea is said to have beneficial properties via lowering iNOS synthesis	Tedeschi et al., 2004
Anti-cancer	Catechin promotes the production and it has been proven to trigger apoptosis, inhibit cellular proliferation, and inhibit oxygenation and movement in numerous carcinoma cells	Singh et al., 2011
Cardio-protective	Tea polyphenols, with their high antioxidant activities, could be potential cardio protective options	Yan et al., 2020

6. Tea polyphenols against various diseases

6.1. Tea polyphenols and kidney stones

According to a large prospective cohort, tea use may protect rather than contribute to the formation of urolithiasis, contrasting with other research findings. Tea is thought to trigger kidney stone formation by interfering with the uptake of oxalate (Demoulin et al., 2022). Tea ingestion was negatively associated with the progression of kidney stones in the Midwives' Health Research, a cohort study of over 81,000 women aged 40–65 years (Salinas-Roca et al., 2022). Furthermore, many systematic evaluations have demonstrated that tea drinking may protect against the development of urolithiasis (Lin et al., 2020; Barghouthy et al., 2021).

6.2. Anticancer properties of tea polyphenols

Tumor divisions have been widely examined *in vitro* for anticancer effectiveness. Ovarian cancer has the greatest fatality rate of any malignant gynecological condition, and theaflavin-3, 3'-digallate (TF3) helps to reduce it. TF3 causes cell death in the OVCAR-3 cell line, which was derived from ovarian cancer (Gao et al., 2019). In rate-3 treated cells, TF3 significantly boosted the production of protease poly phosphatase 1 and activated several isoforms, including casp-3, casp-7, casp-8, and casp-9 (Gao et al., 2019). Caspase-8 stimulates signaling pathways caspases, which then cleave proteins like PARP-1. The pathways that are activated by TF3 are principally due to the decomposition of PARP-1 (Fujimura et al., 2022). Several scientific findings emphasized antioxidant and anti-inflammatory attributes of EGCG, a functional of green tea. Its role as anticancer agent has been established in various scientific experiments but additional research is being carried out for other kinds. Moreover, another vital green tea compound, catechins, proven to alter several biological pathways involved in mitochondria and enhance the anticancer effect of chemotherapy to lessen toxic compounds. In addition to block the clustering of ROS inside the body to inhibit cancer formation, EGCG also inhibits cancer cells proliferation by preventing DNA without any damage of normal body cells. Various studies emphasized on consuming four cups of tea per day for a month to obtain anticancer effects. In addition to that by taking five cups of tea for 6 months' oral cancer can be prevented by deactivating the bacteria involved in it. Beside this it can also suppress oxidative stress and prevent the chances of prostate cancer (Zhang et al., 2022).

Table 2 represents the tea polyphenols and their health properties. When combined with cisplatin, TF3 did not affect normal ovary cells, but it showed a much larger effect on A2780/CP70 and OVCAR3 ovarian cancer cells.

6.3. Antioxidant effects of tea polyphenols

Previous studies discussed the interaction of -epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG) during the transesterification reaction produces TF3 (theaflavin-3,3$'$-digallate), a prominent and abundant component of black tea. As a result, TF3 has been shown to have a variety of therapeutic qualities, like antioxidant capacity and free radical scavenging activity (Qian et al., 2021). As per a recent survey, women who drink much black tea have a lower risk of getting older (Oka et al., 2012). ROS is required for the onset of osteoclasts and also the bone turnover mechanism (Wang et al., 2020). TF3 inhibits osteoclast formation by increasing Nrf2/Keap1 (factors to regulate the homeostasis that controls genes involved in detoxification), signaling along the pathway while decreasing Signaling. A study discovered that oolong tea increased innate efficacy in neonatal heart cells and H9c2 cells by activating Nrf2-regulated mechanisms against JNK-induced hypertrophy (Shibu et al., 2018). Free radical scavenging activity in oolong tea blends was also substantial, with increased antioxidant properties (Cianciosi et al., 2022).

Over the past few decades, scientists have discovered 500 chemical constituents, more than 70 polyphenols with proven antioxidant and beneficial health-boosting potentials. Studies showed that tea with a significant proportion of flavonols has an enhanced antioxidant capacity by stabilizing ROS. Although polyphenols in tea exert antioxidant and anticancer properties, chemotherapy treatment cannot be neglected totally. In synergy, both have proven to be anticancerous (Truong and Jeong, 2022).

6.4. Tea polyphenols and anti-metabolic syndrome

Diabetes mellitus is mostly caused by obesity, and adiposity disorders such as fatty liver, hypertension, and hyperlipidemia are becoming increasingly prevalent. As a result, diabetes and arteriosclerosis may develop. Tea and its phytochemicals may aid in weight loss by hindering fat accumulation (Aloo et al., 2023). On the other side, researchers discovered that drinking black tea polyphenols decreases bad cholesterol levels (LDL, low-density lipoprotein), preventing cardiovascular disease (Ma et al., 2022). It was also discovered that black tea negatively correlated with BMI (Vernarelli and Lambert, 2013). In recent times, significant research has indicated that Tea and its constituents, when consumed daily, may aid in preventing and treating conditions such as hyperglycemia, adiposity, high blood pressure, arteriosclerosis, and carcinoma. Tea is an inexpensive and efficient source of all of these vital phytochemicals. Hence, it is the need of the hour to address concerns like tea ingredient stability, efficacy, and accessibility (Ali et al., 2022d). Several studies showed that functional beverages treat people suffering from hyper cholesterol and hyperglycemia. A recent study revealed that taking green tea after meals three times daily has therapeutic effects in diabetic patients. Other studies revealed that the mechanism involved in countering diabetes by increasing glucose uptake through adipose tissues validates their insulin resistance ability, activating glycogen production in the liver, altering the main enzymes responsible for glucose, and improving insulin emission. A recent study investigated on rats fed with 500 mg/kg of polyphenols extracted from green tea resulted in ameliorated glucose resistance in 1 h. Several studies established tea polyphenols improves lipogenesis and glycolysis (Wang S. et al., 2022).

6.5. Cytotoxic properties of tea polyphenols

Tea polyphenols, like catechins, provide several medical benefits, including preventive medicine and cancer prevention. Women in southern China consume Black tea, particularly green and black tea, and oolong tea, which has reduced the risk of malignant tumors in the southern hemisphere (Lee et al., 2013). In addition, oolong tea phenolic formulations inhibited cell proliferation, DNA breakage, the creation of oligonucleosomal-sized pieces, and the initiation of mediated toxicity in KATO III gastric epithelial carcinoma cells (Das et al., 2021). Additionally, a recent analysis discovered that oolong tea carbohydrates and polyphenols work synergistically to inhibit cancer growth in a sarcoma model while improving antioxidant and immunologic defenses during experimental animals (Fernandes et al., 2020). Anti-proliferative drugs work through signal transduction, and pro-actions *in vitro* activity. The project generated from black tea is commonly regarded as effective for carcinogenesis prevention. Melanoma cells were treated with theaflavin, which caused serious cell death via p53-mediated Proapoptotic, while also molecular markers sheath adhesion and tumor tissue death (Sarma et al., 2020). Likewise, the ROS virtuous cycle predicated on p53 (gene to inhibit tumor development) that tends to increase p53 activity via the p38MARK (p38 mitogen-activated protein kinases) inhibits the Nuclear factor and Transcription factor-dependent rising star enzyme-based Making laws and MMP-9 (Liczbiński and Bukowska, 2022).

6.6. Weight reduction

Overweight is the second most common dietary disease worldwide. According to the Leading Medical Institution's 2010 global survey on diabetes complications, severe obesity claims 2.8 lives each year (Li et al., 2020). The World Health Organization defines obesity as a BMI of 30 kg/m or higher. Obesity has been linked to various medical illnesses, including heart disease, type 2 diabetes, and cancer (Kannel et al., 1991; Knowler et al., 2002; Marmot et al., 2007). This condition is defined by a physiological mismatch between metabolic rate and food intake in a particular body's tissues, which results in excessive fat storage in adipose tissue.

For millennia, black tea flavonoids have been studied for their pro potential. Numerous studies found that black tea polyphenolic compounds can aid in weight loss (Kobayashi et al., 2009; Jin et al., 2013; Wu et al., 2016; Khalid et al., 2023). Tea tannins are shown to affect rat gut bacteria (Chen et al., 2021), which may contribute to green tea catechins' pro effect (Zhong et al., 2006). The hypothesis that black tea influences intestinal flora has only been tested in patients, so no link has been established between black tea's anti-obesity benefits and its effect on gut flora. Polyphenols have been intensively studied for their effects on food intake through neuro-regulatory mechanisms, brain signaling pathways, and external feedback streams (Brindha et al., 2021). According to another study, in obese rats, green tea phenolic acids can reverse the effects of excitatory genes (Truong and Jeong, 2022).

Previous studies showed that a fat diet directly influences gut bacteria physiology resulting in weight gain and GI tract inflammation. In contrast, green tea polyphenols supplements have been proven to prevent dysbiosis and obesity. GTPs work by a principle of strengthening gut bacteria physiology by suppressing the TLR4 signaling pathway and stimulating pro-inflammatory cytokine proteins. Green tea polyphenols strengthen GI track disease resistance and prevent the onset of inflammatory diseases like intestinal bowel syndrome (Li et al., 2020).

6.7. Neuroprotective effect of tea polyphenols

In the average human diet, fruits and veggies are the main sources of phenolic compounds. Bucciantini explored several polyphenol compounds and their therapeutic effects on various human chronic disorders (Bucciantini et al., 2021). This study refers to conventional therapy for ALS and Developmental disorders by using crucial phenolic acids like rutin, carvacrol, and green tea catechins, highlighting the medicinal qualities of polyphenols (Novak et al., 2021). Resveratrol (RSV) may also affect young entry peptide-42 authorization, as per research (Ye et al., 2022) (A42), which has been connected to Alzheimer's disease neurotoxicity, by regulating a leptin enzyme that is still fully biodegradable A4. *In vivo* study, a vital pre-clinical stage, reveals considerable cognitive and memory performance improvement in neurodegenerative models (Zhang et al., 2021). Phenolic in the diet has now been linked to improved cognitive function, as per a comprehensive assessment of the data, and their administration may help avoid cognitive deterioration (Khalatbary and Khademi, 2020). The potential of these chemicals in preventing and fighting neuronal illnesses has recently been addressed (Spencer, 2009a,b; Zhao, 2009). Similarly, Phenolic is shown to slow the progression of several neurodegenerative disorders associated with cognitive impairment, including Alzheimer's disease, Parkinson's disease, dementia, and musculoskeletal problems (Hamaguchi et al., 2009; Ardah et al., 2014; Freyssin et al., 2018).

6.8. Polyphenols and neurodegeneration

Neurodegeneration refers to the gradual deterioration of neural functioning, particularly neuronal loss. High blood pressure, Parkinson's, Prion ailments, nerve diseases, Huntington's disease, corticospinal ataxia, and neuroinflammation disorders are all examples of insanity (Ahmad et al., 2017). Alzheimer's disease is the most common form of dementia, and it is associated with a decrease in cranial capacity, which causes significantly fewer cognitive impairments than functional brain aging (Ray and Davidson, 2014). Dementia, cognitive disability, and cognitive impairment are all cognitive disorders. Alzheimer's disease is influenced by a complex mix of hereditary and environmental variables, such as medical history and eating habits (Ray and Davidson, 2014). Different mental and related conditions often deteriorate with aging. Various neurobehavioral skills are lost as we age, as are procedural and fiction memory (Ren et al., 2013), but also decreased reaction inhibition and concentration on elevated activities (Di Meo et al., 2020).

Several studies depicted the preventive effects of polyphenols toward various neurotoxic parameters, which include memory neurotoxic elements etc. These changes resulted in hippocampal cell loss, decreased internal memory, and basal forebrain cholinergic neuron atrophy (Fernandes et al., 2021). The effect of flavonoids on time-of-life of life nerve activity has only lately been thoroughly studied, and multiple *in vitro* are accessible (Table 3). As per a new study, RSV significantly reduces the number of interleukins, particularly IL-1 and IL-6, correcting for chronic activity (Magrone et al., 2019).

Moreover, by interrupting the latent nuclear constructs of texts from sense organs, flavonoids can obstruct main product routes. Likewise, to slower cognitive decline, some research looked at how polyphenol affects motor dysfunction, which has been connected to neurodegenerative disorders and brain aging. Tea tannins have been shown in Parkinson's disease baboons to alleviate axonal damage caused by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Azam et al., 2019). Figure 4 shows the neuroprotective pathway. After a 6-month diagnosis and a prebiotic fiber Aegean diet, recollection improves, concentration, executive abilities, and optical vision, according to a 2015 study (Knight et al., 2015). Likewise, a 6-year drug study involving 447 participants discovered a link between the lignan diet and a big decline in psychiatric illness (Varga et al., 2020). In addition to diet trials, several investigations found that polyphenol-based nutraceutical therapy improved learning ability.

In this context, there is evidence that grape and blueberry extract consumption significantly impacts mental performance in healthy young people over each test (Philip et al., 2019). Much research has been done to probe the organic antioxidant effects of olive flavonoids such as glycosides, tocopherol, carotenoids, thymol, and others (Annunziata et al., 2021). Resveratrol at 10 mg/kg reduced clinical symptoms' thickness dramatically in male adult CD1 mice just under a week after intracoronary fluid resuscitation by lowering IL-1 speech, a key cytokine (Afzal et al., 2022). Table 4 offers more examples of polyphenols having neuroprotective effects reported *in vitro*. The foremost anticipated goals are to tremendously aggravate' neuroprotective.

TABLE 3 The most important *in vitro* data for polyphenol effects on age-related brain changes.

Polyphenols	*In vitro* pathway	References
EGCG	Increases cell viability by suppressing caspase activity and decreasing malondialdehyde levels	Choi et al., 2001
Phenolic from Arbutus unedo	Increases autophagosome and intracellular space refresh rate of autophagic protein denaturation while decreasing peroxidation	Macedo et al., 2018
Phenolic extricate Ginkgo biloba	Rises CREB onset and activates the BDNF scheme	Hou et al., 2010
Curcumin	Aged excitability is suppressed by increasing M2 astrocyte disunity and enhancing the toll-like nicotinic 4/Nuclear factor signaling pathways	Zhang J. et al., 2019; Zhang Z. et al., 2019

6.9. Neuro-inflammation

Many correlating irregularities discovered during neurodegeneration have been linked to low-level autoimmune conditions, stimulation of sources on the topic, and glia known as neurons. Several innovative functional products in the market have been fortified with compounds extracted from vegetables and fruits (Ahmed et al., 2021). Most importantly, plant-based secondary metabolites considerably polyphenols, are crucial in combating inflammation, diabetes, and oxidative stress-related disorders. Previous studies revealed plant-based secondary metabolites' role in regulating gastrointestinal mechanisms. Noticeably, the action mechanism was completely different in both *in vivo* and *in vitro* studies. This difference is directly linked to the GI track mechanism that conducts the compositional changes in these metabolites, modifying their action role in the digestive system (Wojtunik-Kulesza et al., 2020). Foods high in specific forms of secondary polyphenolic phytochemical constituents, For example, the striving of altering nervous responses have garnered praise (Rangarajan et al., 2016; Khalid et al., 2022a). Lately, turmeric, a diarylheptanoid, and epicatechin were suggested as key treatments for immunologic infections associated with hypertension (Chen et al., 2022). 400 mg of curcumin (Longvida®) medicinal additives improved mental function in healthy aged persons in a controlled, double-blind and moderate trial. Curcumin inhibited gelation of IL-1, TNF-, vasodilators E2, peroxynitrite, inducible NO glucanase,

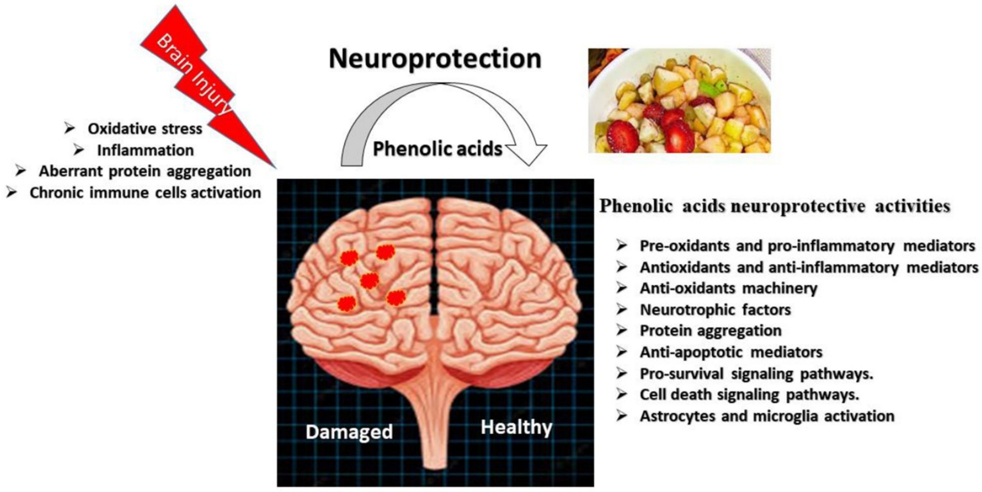

FIGURE 4
Phenolic acid neuroprotective mechanism.

and Lipoxygenase *in vitro* experiments (Kang et al., 2022). Crocin (a carotenoid), oral dosages (an alkaloid), scutellarin (a flavone), but also anthocyanins a flavanone-7-O-glycoside are phenolic acids affiliated with predicament properties (Revi and Rengan, 2021). In patients, a 1 g numerous times oral mouthful of Rosuvastatin, a SIRT1 activator, reduced the matrix process is driven, raised myocyte interleukins, IL-4, but also fibroblast economic expansion (FGF)-2, and inevitably energized host defenses (Hao et al., 2022).

6.10. Autophagy

Fermentation is an ATP-dependent natural process that engages in a variety of cell procedures such as load transfer and breakup, as well as recycling inside of autophagy. There is event of a food shortage, this included junctional retooling, cellular protein degradation or amino acid formulation, and pro corridor senior execs (Giampieri et al., 2019). Autophagy is necessary for the advancement of some diseases, such as Alzheimer's, Parkinson's, and Huntington's. These diseases are distinguished by abnormal peptide aggregate and deposit or abnormal unfolded membrane proteins as a result of decreased turnover or rapid protein depositing, resulting in progressive neuronal death (Tang et al., 2022).

This same autophagosome phase is important in measuring the quantity of such cumulative enzymes, such as open beta atherosclerotic plaque and tau throughout Parkinson's disease, that are bred in captivity in Parkinson's disease, but also various huntingtin in Parkinson's disease, whose approval is slowed when the aging process is broken (Xiao et al., 2022). An autophagosome process is important in many genetic diseases, along with Parkinson's, Parkinson's, but also Huntington's disease. These illnesses are distinguished by increased peptide aggregation and deposit and abnormal unfolded proteins resulting from decreased turnover or faster protein deposit, which results in neuronal death (Wu et al., 2021). In this reference, the endosomes phase is necessary for lowering the levels of such combined proteins, which include amyloid plaques and tau (essential enzyme regulates the basic structure of nerve cells)in hypertension, which are biologically mutants in Parkinson's disease and Huntington's disease, or whose approval is slowed once peroxidation is inhibited (Yang et al., 2021).

TABLE 4 List of neuroprotective polyphenols.

Phenolic	The method in cultured cells	References
Que, genistein	Top player cytokine expression (IL-6, TNF-, IL-1, and COX2) is reduced	Spagnuolo et al., 2018
Que	The chemopreventive enzymes paraoxonase 2 and Nrf2-ARE are activated (PON2)	Costa et al., 2016
Corylin	In LPS-activated rodent model types axonal cells, the production of NO and rising star middlemen TNF-alpha, IL-1, and IL-6 is lowered (BV2 cells)	Huang et al., 2018
Celastrol	The H661 cell is being used to test the impact of Oxidase protease inhibitory activity on peroxidation due diligence	Whitehouse et al., 2016
Caffeic acid	Endoplasmic stress and spontaneous death in granulosa cells neurons in culture	Taram et al., 2016
Butein	Suppression of IB kinase, inactivating cyclooxygenase, but also reactive oxygen inhibit NF-B	Padmavathi et al., 2017

6.11. Vascular function

Not only must the arteries within the brain be fit and active to prevent ischemia, but they must also be nutritious to maintain perfusion, which improves mental capacity. Endothelial failure is associated with the onset and advancement of metabolic disorders, which can contribute to cognitive loss and neurotoxicity as we age (Wu et al., 2022). Flavones can also reduce the activity of nitric oxide synthase, lowering the need for NO during vascularization (Xiao et al., 2022). An increase in NO relaxes the muscle that brings the blood, promoting vascularization and neurogenesis (Grosso et al., 2022).

Biological membranes discharge abnormal unfolded proteins and harmful molecules that activate monocytes in neurodevelopmental disorders, culminating in a normal brain response that can impede microcirculation. Several experiments have been conducted to investigate the effect of polyphenolic compounds on methods used and bond strength intermediaries, which may aid in reducing the effects of autoimmunity on the endothelium. Ingesting chemicals, caffeic acid, and RSV drastically decreased the inflammatory reaction within the vascular wall, such as pro-inflammatory cytokines protein 1, monocytes inflammatory proteins 1, MIP-1, chemokine receptor-1, and Kinases pro-inflammatory cytokines receptor-1 (Rajan et al., 2022). Green tea polyphenols have proven to ameliorate the ROS activity of the blood cells, inhibiting the oxidation of LDL cholesterol hence lowering the chances of cardiovascular disorders. Studies revealed that daily consumption of 5 or more tea cups lowered the death rate to 31% in women suffering from CVD and heart failure. CVDs like hypertension, stiffness of arteries, endothelial malfunction, cardiomyopathy etc. have been linked to increased oxidative stress. An *ex vivo* and *in vitro* research conducted by Li et al., summarized that tea polyphenols exert lowering impacts in hypertensive patients by relaxing the muscles, preventing renin activity, and boosting endothelial nitric oxide synthase function and anti-inflammatory impact on vessels. According to another study, CVD's can be controlled to levels of 10 mg/dl (0.25 mmol/L) of LDL, by consuming 1 capsule of theaflavin-enriched GTE (375 mg) regularly and 2 cups of green tea.

6.12. Oxidative stress

Enhanced oxidative stress is linked to neuronal injury in neurodegenerative diseases. When T lymphocytes are overstimulated, they are among the primary producers of radical precursors and contribute to oxidative stress. Tea Phenolic have protective properties that can both protect cells and slow the progression of toxicities. Throughout this entire reference, the effects of curcumin on the health of Mongolian ferrets with temporary carotid obstruction were studied (Song et al., 2021). Curcumin submucosal transfusions or food consumption reduced the enhanced lipid, peroxides, and apoptotic markers caused by myocardial damage. These protective benefits were connected to reducing glial activation, which led to the lower inflammatory and anti-mediator release. According to previous studies, various other ailments are stimulated by oxidative stress. Nrf2 is a gene that inhibits oxidative stress by stimulating enzymes involved in the mechanism, like glutathione peroxidase, heme oxygenase-1 and malondialdehyde (MDA). These enzymes play a crucial role by disintegrating the structure of free radicals, preventing the oxidation of vita cells (Khayatan et al., 2022).

In an induced cytotoxicity cognition loss scenario in Swiss male albino mice, hesperidin treatment dramatically ameliorated cognitive loss (Fakhri et al., 2022).

7. Conclusion

This review led us to the conclusion that polyphenols may be good for human health due to their antibacterial, anticancer, anti-inflammatory, anti-apoptotic, and antioxidant properties. Tea is naturally rich in bioactive compounds that boost health. Tea polyphenols are frequently extracted using different solvents by conventional and advanced mechanisms. In conventional methods, hot water extraction is more efficient than cold water extraction. Innovative techniques like ultrasound, microwaves, and air pressure can be implicated in more effective and efficient teal polyphenol extraction. Although, each extraction process has its limitations. It is recommended to conduct future research to optimize more specific extraction techniques with better efficacy. In this review, we also demonstrated numerous recent research to enlighten the significance of different teas, their chemical composition and theirhealth-promoting role by preventing and delaying metabolic illnesses, notably neuroprotection. Tea polyphenols are the major constituents possessing antioxidant, anti-diabetic, and anti-inflammatory attributes. Recent investigations have also suggested the possibility that phenolic acids have neuroprotective effects that target a variety of cellular pathways to prevent the development of cognitive problems. Due to potential safety profiles and immunomodulatory effects, tea might provide a new source for chemopreventive or therapeutic agents for various chronic diseases. Up to now, great efforts have been made both *in vitro* and *in vivo*, but the precise mechanisms are still unclear. Therefore, more carefully-designed studies are needed to deeply elucidate the immune-potentiating properties toward cellular and humoral immune responses.

8. Future research suggestion

During the past few decades, tea has been consumed in several forms for its relaxing and therapeutic potential. Considerably, due to its health-boosting functions, tea has been proven to aid in preventing several ailments by stopping the activation and progression of metabolic syndromes, various types of cancers, degenerative disorders, CVD's, anti-inflammatory, anti-diabetic, weight reduction, anti-hypertensive effects. This review summarized that there had been enough evidence for the beneficial functions of tea, but a few researchers also discuss the health safety issues of tea and its by-products. Hence, advanced research should be conducted on tea and its by-products bioavailability, its synergic impact along with other medical treatments and supplementation on various diseases and further need to explore the action mechanism of tea and its products. However, tea consumption as a refreshment and therapeutic function still been validated as safe and endorsed until now.

Author contributions

HS and AAl: conceptualization. HS, AAl, YZ, AAh, SR, and AK: writing—original draft preparation. HS, AAl, AAh, SR, TM, and AK: writing—review and editing. YZ and HQ: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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

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Evaluation of different *Terminalia chebula* varieties and development of functional muffins

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Terminalia chebula is a valuable medicinal plant that can be used in human nutrition. The current study was conducted on different varieties of *Terminalia chebula* (Harad) and aimed to investigate their proximate composition, antioxidant activity (DPPH and FRAP assay), and total phenolic content (TPC) by using different solvents (ethanol, acetone, hexane, and distilled water) for further use in the development of functional muffins. Wheat flour was supplemented with the Harad varieties (at 5%, 7.5%, 10%, or 15%) for the preparation of muffins. The color tonality (L*, a*, b*, C*, and h*) of the final product was measured with a digital colorimeter, and the sensory analysis was performed by using a 15-point scale. The results showed that the DPPH values of the Harad samples varied from 22.16 ± 1.27 to 84.33 ± 1.29, while the FRAP values ranged from 18.65 ± 1.73 to 113.4 ± 2.1 mmol FeSO4/g, depending on the solvent type. TPC in different Harad varieties ranged from 10.77 ± 2.35 to 107.20 ± 1.80 mg GAE/100 g, whereas the total flavonoid content ranged from 3.78 ± 0.03 to 47.91 mg QE/100g. Among Harad varieties, the Kabuli variety contains the highest amount of Ca, Na, and K, namely 95.58, 2.16, and 113.66 mg/kg, followed by the black variety with 89.58, 1.83, and 105.08 mg/kg respectively. The muffins prepared with black Harad give the highest values of the color parameters compared to other samples. According to the consumer's choice, the sensory profile assessment revealed that the muffins supplemented with 7.5% of different Harad varieties (green, black, and Kabuli) were the most acceptable. Therefore, Harad varieties could partially replace the white flour in muffins to improve their nutritional value. These results can be useful for the bakery industry interested in developing new functional foods.

KEYWORDS

Harad, antioxidant potential, medicinal plant, wheat muffins, Principal Component Analysis

1. Introduction

Herbal medicine gained popularization over the years, and it was the base of the modern medicine's development. According to a survey, 70–80% of the world population directly or indirectly depends on medicinal herbs for primary health care and treatments (Panda et al., 2019; Ranjha et al., 2020; Shehzadi et al., 2020; Sabtain et al., 2021). The plants are used in several medicinal systems in many countries and are natural and available cheap sources of bioactive compounds with benefits in chronic disease prevention and treatment (Panda et al., 2019; Ranjha et al., 2020; Shehzadi et al., 2020; Sabtain et al., 2021). To make the best use of phytoconstituents, their extraction from plant materials (fruits, seeds, peels, leaves, etc.) is necessary (Ranjha et al., 2021; Sabtain et al., 2021; Nadeem et al., 2022).

Scientists screening many medicinal plants discovered that one of the most well-regarded medicinal plants (Mahmood, 2019; Shehzadi et al., 2020) is *Terminalia chebula*, called also Harad, or Chebulic Myrobalan. *T. chebula* contains many different kinds of phytoconstituents that unveiled a great number of medicinal activities. The myrobalan fruit has various health merits, being used as a household remedy against numerous human illnesses since ancient times (Majed et al., 2015; Van Wyk and Wink, 2018).

Globally, *T. chebula* has been identified in tropical regions (Saleem et al., 2002). It is a wild deciduous plant native to south Asia from India, Iran, Pakistan, Burma, southwest China, Vietnam, and Malaysia (Gaire and Kim, 2014; Sadeghnia et al., 2017). In Pakistan, there are three different varieties, namely green, black, and Kabuli, possessing different shapes and sizes. Mostly, the outer skin (pericarp) is used in different pharmacological preparations. *T. chebula* is cultivated from seed during the spring season in different soil types, from clay to sandy (Sharma et al., 2011).

In many food products, *T. chebula* is a functional ingredient because it plays a pharmacological role (Sharma et al., 2011). The *T. chebula* comprises therapeutic constituents such as chebulic acids (Lee et al., 2010), ellagic acid, chebulanin (Chattopadhyay and Bhattacharyya, 2007), gallic acid (Patel Madhavi et al., 2010), luteolin (Kumar, 2006), rutin, ethaedioic ethanoic acid, ethyl gallate, tannic acid (Juang et al., 2004), ascorbic acid, quercetin, neochebulinic acid (Han et al., 2006), ethaedioic acid (Reddy et al., 2009), corilagin, terpinenolsterpineol, terpinenes (Srivastav et al., 2010) chebulinic acids, casuarinin, 2,4-chebulyi–β-D-glucopyranose (Juang et al., 2004) 1, 6-di-O-galloyl-D-glucose, 3,4,6- tri-O-galloyl-D-glucose, 1,2,3,4,6-penta-O-galloyl-D-glucose, punicalaginterflavin-A, terchebin, anthraquinone (Srivastav et al., 2010). Moreover, it is recognized as having anti-inflammatory, antioxidant (Suchalatha and Devi, 2005), antiviral (Kim et al., 2001), antidiabetic (Rao and Nammi, 2006), hepatoprotective (Vidya et al., 2011), antibacterial (Kannan et al., 2009), antiviral (Kim et al., 2001), antifungal (Sahab and Sabbour, 2011), antiulcerogenic (Sharma et al., 2011), antinociceptive (Kaur and Jaggi, 2010), immunomodulatory (Aher and Wahi, 2011), radioprotective (Jagetia et al., 2002), cardioprotective (Suchalatha and Devi, 2005), anti-hyperglycemic (Shreedevi and Sampathkumar, 2011), antiplasmodial (Prakash et al., 2013), anti- arthritis (Seo et al., 2012), hypocholesterolemic (Maruthappan and Shree, 2010) and anticancer (Saleem et al., 2002) activities.

The interest of consumers for functional foods is increasing, and the use of medicinal plants in their development is an opportunity. The influence of such an ingredient on the quality of the final product should be considered in order to obtain the maximum health benefits, without affecting product's quality. To our knowledge, there are no studies regarding the effects of different Harad varieties on the physical and sensory characteristics of muffins. Furthermore, the characterization of the Harad varieties justifies the possibility to include them in a baked product like muffins, to create a novel functional food. In this research, different varieties of *Terminalia chebula* were investigated, aiming to develop novel functional muffins by replacing wheat flour in various amounts. For this purpose, the proximate analysis was performed to compare the three varieties (green, black, and Kabuli) of *Terminalia chebula,* along with the TPC, DPPH, and FRAP employed to measure the phenolic and antioxidant contents, respectively. The color tonality (L*, b*, C*, a*, and h*), sensory texture, and other sensory parameters were determined in order to check the quality and consumer acceptance of muffins supplemented with *Terminalia chebula*.

2. Materials and methods

2.1. Materials

The present research was accomplished in the postgraduate laboratories of the Food Science and Technology Department at Bahauddin Zakariya University, Multan, Punjab, Pakistan. Different varieties of Harad (*T. chebula*) were procured from the Al-Hamad Pansare store, Multan. The raw materials for muffin preparation were bought from the local market. At the same time, the analytical grade chemicals were purchased from Merck and Sigma-Aldrich. The different varieties of Harad were manually removed from inessential materials. The gall or fruit particle size was reduced into fine powder through the local grinder and packed and stored at 37°C for further analysis.

2.2. Harad varieties characterization

2.2.1. Proximate analysis of different varieties of Harad

Different varieties of Harad were evaluated for proximate analysis in terms of crude protein, moisture, ash, crude fat, crude fiber, and nitrogen-free extract (NFE), according to their standard methods described by the AOAC (2016). NFE was calculated using the following equation (Eq. 1):

$$NFE\,(\%) = 100 - \begin{pmatrix} moisture + ash + crude\ protein \\ + crude\ fat + crude\ fiber \end{pmatrix} \quad (1)$$

2.2.2. Quantification of minerals

Macro and microelements from *T. chebula* varieties (green, black, and Kabuli) were measured by using a Flame photometer and atomic absorption spectrophotometer according to the method presented in the AOAC (2016). The sample was digested with nitric acid (HNO_3) and perchloric acid ($HClO_4$). After that, it was diluted with de-ionized water and analyzed for specific elements by using a standard calibration.

2.2.3. Antioxidant potential of *Terminalia chebula* varieties

2.2.3.1. Preparation of the extract

The extracts were prepared with different solvents such as ethanol, acetone, hexane, and distilled water at a 1:10 ratio of powder: solvent. The samples stayed overnight at room temperature (about 25°C), then they were shacked at 280 rpm for 5–8 h, filtered with Whatman filter paper no.1, and evaporated with a vacuum rotary evaporator (Heidolph, Hei-Vap, Germany) at 20°C. Then, the extract was centrifuged at 3,000 rpm for 5 min. Finally, the concentrated extract was stored in 50 mL Falcon tubes at 2–8°C.

2.2.3.2. Total phenolic content determination

The total phenolic content (TPC) of *T. chebula* was determined according to Singleton et al. (1999) method, with slight modification. The extract reacted with 2.5 mL of 10 times concentrated Folin Ciocalteu Reagent (FCR) and 2 mL of 7.5% Na_2CO_3 for 30 min in darkness. The different extract samples were measured at 760 nm wavelength using a spectrophotometer and a standard gallic acid solution with 10–100 mg/L concentration.

2.2.3.3. DPPH assay

The free radical scavenging activity was evaluated trough DPPH assay, according to Brand-Williams et al. (1995) method with little modifications. The different extracts of *T. chebula* ranging between 50–100 μL were diluted up to 100 μL with methanol. A quantity of 2 mL of Harad extract with 3 mL DPPH was incubated for 30 min in a shaded place at room temperature. The calibration standard ascorbic acid was used for measuring free radical scavenging activity at an absorbance of 517 nm (Eq. 2).

$$DPPH\ scavenging\ activity\,(\%) = \frac{A_B - A_A}{A_B} \cdot 100 \qquad (2)$$

Control A_B is the absorbance of the control, and Sample A_A is the absorbance of the tested samples.

2.2.3.4. Total flavonoids contents

The total flavonoid content (TFC) was measured by means of the method described by Dewanto et al. (2002). An amount of 0.3 mL of $NaNO_2$ (5%) solution was mixed with 3 mL plant extract. Then, 0.6 mL of $AlCl_3$ (10%) solution was added to 2 mL of (1 M) NaOH solution after 5 min. The solution was vortexed and measured at 510 nm wavelength using a spectrophotometer. The TFC was expressed in mg of quercetin equivalent (mg of QE).

2.2.3.5. FRAP assay

The ferric-reducing antioxidant potential of *T. chebula* extract was measured by using Zahin et al. (2010) with slight modifications. The FRAP reagent comprised 10 mM/L TPTZ solution, sodium acetate buffer solution, and 20 mM/L ferric chloride at a 1:1:10 ratio, at pH of 4.0. A quantity of 100 μL of *T. chebula* extract reacted with 2 mL of FRAP regent and then it was placed in a dark area for 20–30 min at 37°C. The ferrous sulfate solution with a concentration ranging from 100 to 1,000 μM was used for standard calibration and the absorbance was measured at 593 nm with a spectrophotometer. The results were expressed as μM of Fe (conversion of ions).

2.3. Muffins development

Muffins were prepared by replacing wheat flour (WF) with *T. chebula* of different varieties (green, black, and Kabuli) in various amounts (Table 1). Firstly, a foam was formed from eggs and sugar, then the powder ingredients were added to the foamy mixture to make the batter. The batter was put into muffins shape holding dish and baked at 149–200°C in the baking oven for 20–25 min. After that, the muffins were cooled at room temperature and kept in a polythene zipper bag for further analysis.

2.4. Muffins characterization

2.4.1. Physical analysis

The muffins color was determined with a Chroma meter CR-400 (Konica Minolta INC. Japan). An amount of 55 g of muffins was taken, and the color values in terms of L* (Lightness), a* (+ redness, − greenish), and b (+ yellowness, − blueness) were measured. The C* (Chroma) and h* (Hue angle) were calculated according to Gouveia et al. (2007).

2.4.2. Sensory profile evaluation

The muffins were subjected to sensory evaluation by a trained panel from the Institute of Food Science and Nutrition (student and faculty members: aged from 20 to 55 years) BZU, Multan, Punjab, Pakistan. The total number of panelists was 30. A quantity of 55 g of muffins from each variety and with various addition levels of *T. chebula* was served at room temperature to the panelists. The *T. chebula* level used from each variety was unknown by the panelists who were asked to evaluate and record crust, crumb color, and

TABLE 1 Preparation of wheat and Harad (*T. chebula*) powder (green, black, and Kabuli) supplemented muffins.

Treatments	Wheat Flour (%)	Green (%)	Black (%)	Kabuli (%)
T_0	100	0	--	--
T_1	95	5	--	--
T_2	92.5	7.5	--	--
T_3	90	10	--	--
T_4	85	15	--	--
T_5	95	--	5	--
T_6	90	--	10	--
T_7	92.5	--	7.5	--
T_8	85	--	15	--
T_9	95	--	--	5
T_{10}	92.5	--	--	7.5
T_{11}	90	--	--	10
T_{12}	85	--	--	15

appearance, volume, aroma, mouth feel, overall acceptability by using the 15-points scale method (Meilgaard et al., 1999). Three replications were made. The presentation order of the samples was randomized depending on the addition level and positional codes. Mouth palletizer water and crackers were provided to panelists to rinse their mouths between evaluations. Panelists were accommodated in different booths equipped with bright white lighting in a separate room for each assessment.

2.4.3. Sensory textural analysis

The textural characteristics (softness, hardness, springiness, and gumminess) of muffins were determined by sensory evaluation, as described in section 2.4.2.

2.5. Statistical analysis

Each parameter was attained by using complete randomized design (CRD) and analysis of variance (ANOVA) for all treatments, at significance levels of $p \leq 0.01$ and $p \leq 0.05$ with Statistix 8.1 software. Duncan's preference test was used to differentiate between the treatments. Principal Component Analysis was carried out using XLSTAT software, 2022 version.

3. Results and discussion

3.1. Harad varieties characterization

3.1.1. Proximate analysis

The proximate analysis of three varieties of *T. chebula* (green, black, and Kabuli) showed significant differences ($p < 0.05$; Table 2). The moisture content of Kabuli sample was lower (8.64%), while the green variety contained the highest water content (10.55%). The ash content of the Kabuli variety was the highest, 4.18%, with the lowest ash content being attained by the green sample (3.45%).

The highest fat content was recorded by Kabuli sample (2.25%), while the black one had the lowest value (1.54%). The amount of crude protein in *T. chebula* varieties ranged from 3 to 5%. The fiber content varied from 6.25% in Kabuli to 5.37% in the green variety. The NFE content was the highest in the black sample (75.98%), followed by 74.91% in green *T. chebula*, and 75.67% in Kabuli variety. Lwin et al. (2020) and Hussain et al. (2009) reported that the moisture, fat, protein, ash, fiber, and carbohydrate of *T. chebula* was 8.65, 1.45, 3.78, 2.68, 19.33, and 83.43%, respectively. The differences regarding the proximate composition of Harad varieties could be attributed to the phenotypic, genotypic and environmental variations (Navhale et al., 2011).

TABLE 2 Proximate composition of different varieties of Harad (*T. chebula*).

Parameter	Green	Black	Kabuli
Moisture (%)	10.55 ± 0.27^{a}	9.66 ± 0.12^{b}	8.64 ± 0.15^{c}
Fat (%)	1.88 ± 0.11^{b}	1.54 ± 0.07^{c}	2.25 ± 0.1^{a}
Protein (%)	3.84 ± 0.07^{b}	3.24 ± 0.11^{c}	4.53 ± 0.37^{a}
Fiber (%)	5.37 ± 0.31^{c}	5.69 ± 0.17^{b}	6.25 ± 0.61^{a}
Ash (%)	3.45 ± 0.13^{c}	3.89 ± 0.11^{b}	4.18 ± 0.13^{a}
NFE (%)	74.91 ± 0.25^{b}	75.98 ± 0.15^{a}	74.15 ± 0.13^{c}

The different letters on the same line show a significant difference according to Duncan's test ($p < 0.05$). NFE, Nitrogen-Free extract.

TABLE 3 Mineral profiling of the Harad varieties (*T. chebula*) in (mg/kg).

Minerals	Green	Black	Kabuli
Ca	81.66 ± 1.12^{b}	89.58 ± 7.60^{ab}	95.58 ± 3.81^{a}
Na	1.25 ± 0.90^{b}	1.83 ± 0.38^{ab}	2.16 ± 0.38^{a}
K	101.58 ± 1.50^{b}	105.08 ± 2.50^{b}	113.66 ± 2.26^{a}
Pb	0.36 ± 0.06^{b}	0.48 ± 0.07^{b}	0.67 ± 0.05^{a}
Zn	0.86 ± 0.06^{ab}	0.78 ± 0.04^{b}	0.95 ± 0.04^{a}
Fe	7.49 ± 1.25^{b}	18.83 ± 2.9^{a}	15.54 ± 0.26^{a}
Ni	0.79 ± 0.18^{ab}	0.91 ± 0.03^{b}	0.96 ± 0.06^{a}
Cu	0.51 ± 0.31^{ab}	0.94 ± 0.04^{a}	0.75 ± 0.05^{b}
Cd	0.14 ± 0.08^{a}	0.08 ± 0.02^{ab}	0.15 ± 0.009^{b}

The different letters on the same line show a significant difference according to Duncan's test ($p < 0.05$).

3.1.2. Mineral analysis

The results revealed that the Kabuli variety of *T. chebula* contains the highest amount of Ca, Na, and K, namely 95.58, 2.16, and 113.66 mg/kg respectively, followed by the black variety with 89.58, 1.83, and 105.08 mg/kg, respectively (Table 3). The lowest value of Ca, K, and Na was recorded in the green variety, ranging from 81.66, 101.58 to 1.25 mg/kg, respectively. The micro-elements, such as Pb and Zn, were in the highest amounts in Kabuli variety (0.67 and 0.95 mg/kg, respectively). At the same time, black variety contains a higher concentration of Fe, with 18.83 mg/kg, than the other two varieties.

Some trace metals or heavy metals, like Ni and Cd, were higher in Kabuli variety compared to the others, with values of 0.96 mg/kg and 0.15 mg/kg, respectively. In comparison to other varieties, the Cu content was higher (0.94 mg/kg) in black Harad of *T. chebula* (Table 3). The current concentration is similar to that reported by Hussain et al. (2009). The differences among varieties regarding the minerals content could be due to the plant genetics, location, climate and/or environmental factors (Waheed and Fatima, 2013). Furthermore, the high concentration of some elements could be also attributed to their uptake from the soil and water (Waheed and Fatima, 2013).

3.1.3. Antioxidant potential of *Terminalia chebula* varieties

3.1.3.1. Total phenolic content

The results of the total phenolic content revealed that the green variety contained a higher value of TPC (79.10 mg GAE/100 g) compared to the other varieties' extracts in acetone and ethanol. In contrast, Kabuli variety exhibited the lowest value for TPC in ethanol, while the black *T. chebula* variety exhibited the highest TPC in water and the lowest in hexane and acetone (Table 4). The results showed that for the green variety, the best solvent was ethanol (71.31 mg GAE/100 g), while for the black and Kabuli variety water extraction is recommended since the greatest amount of TPC was obtained. Rekha et al. (2014) and Birur et al. (2015) reported that the range of total phenolic content was 21–107 mg GAE/100 g d.w. in different extract

TABLE 4 Antioxidant activity of different varieties of Harad.

Solvents	Variety	Total Phenolic Content (mg GAE/100 g)	Total Flavonoids Content (mg QE/100 g)	Antioxidant Activity	
				DPPH (%)	FRAP (mmol/100 g)
Ethanol	Green	71.32 ± 1.31[c]	31.11 ± 0.91[c]	38.16 ± 1.91[g]	64.50 ± 2.20c
	Black	54.74 ± 1.87[d]	22.17 ± 0.11[d]	49.87 ± 0.89[f]	56.54 ± 1.78[d]
	Kabuli	32.36 ± 1.83[f]	13.12 ± 0.04[f]	22.16 ± 1.27[h]	41.62 ± 2.20[f]
Acetone	Green	79.10 ± 1.92[b]	34.5 ± 1.21[b]	80.68 ± 0.89[b]	73.41 ± 1.71[b]
	Black	31.53 ± 1.96[f]	12.71 ± 0.81[f]	48.61 ± 1.21[f]	44.56 ± 1.85[f]
	Kabuli	42.32 ± 2.08[e]	17.09 ± 0.56[e]	54.16 ± 0.84[e]	55.78 ± 3.32[d]
Hexane	Green	12.55 ± 2.27[gh]	4.08 ± 0.04[gh]	82.55 ± 0.61[ab]	32.54 ± 2.12[g]
	Black	10.77 ± 2.35[h]	3.78 ± 0.03[h]	47.51 ± 1.20[f]	18.65 ± 1.73[h]
	Kabuli	14.54 ± 1.53[g]	5.66 ± 0.1[g]	59.54 ± 1.56[d]	27.48 ± 1.95[i]
Water	Green	53.69 ± 1.62[d]	23. 56 ± 1.11[d]	84.33 ± 1.29[a]	51.62 ± 0.32[e]
	Black	107.20 ± 1.80[a]	47. 91 ± 1.82[a]	72.88 ± 4.54[c]	113.4 ± 2.1[a]
	Kabuli	72.49 ± 1.75[c]	31.35 ± 1.06[c]	59.17 ± 0.88[d]	76.33 ± 1.81[b]

The different letters on the same column show a significant difference according to Duncan's test ($p < 0.05$).

(ethanol, acetone, water, hexane, and methanol). Similarly, Khan et al. (2014) showed that the TPC in green and black varieties was 292.78 and 323.5 mg GAE/100 g, respectively. The efficiency of a certain solvent is driven by the polarity of the phenolic molecules, being known that polyphenols are more soluble in organic solvents because of the hydroxyl groups contained (Hassan Bulbul et al., 2022). The differences compared to the existing literature can be due to the presence of some carbohydrates, terpenoids, or ascorbic acid, or extraction protocol and/or the geographical origin of the plants (Aryal et al., 2019). It has been reported that *T. chebula* fruit contains 14 phenolic acids derivates such as caffeic acid, digallic acid, ellagic acid, ethyl gallate, eugenol, ferulic acid, gallic acid, melilotic acid, methyl gallate, p-coumaric acid, phloroglucinol, pyragallol, vanillic acid, and 4-O-methylgallic acid (Poudel et al., 2023). The polarity of solvents is a key factor in TPC extraction, being known that the relative polarity of the solvents is ranked as water > ethanol > acetone (Zhu et al., 2020). Thus, compared to the other solvents, acetone would have the worst extraction efficiency of TPC. However, our results do not confirm this hypothesis, a fact that was also supported by other studies and that proved the complex influence not only of the solvent type, but also of the chemical structure and physical properties of the sample (Zhu et al., 2020).

3.1.3.2. Total flavonoids content

The green Harad variety has the highest flavonoid content (34.5 mg QE/100 g) for the acetone extract, while the Kabuli and black varieties showed the highest values (31.35 and 47. 91 mg QE/100 g respectively) for the water extract (Table 4). Among the studied sample, the green one was proven to possess the greatest flavonoid content in ethanol and acetone, while in water the black sample had the most abundant TFC. Vardin and Yilmaz (2018) affirmed that the TF content of *T. chebula* can provide up to 88 mg QE/g pulp portion (dry weight). The antioxidant properties of the flavonoids which are secondary metabolites from plants depend on the number and position of the free -OH groups, while the variations in flavonoid content depend on genetic factors, and environmental conditions (Aryal et al., 2019). The literature reported that *T. chebula* contains 6 most important flavonoids like 3,4′-dimethoxy quercetin, 3-methoxy quercetin, isoquercetin, luteolin, quercetin and rutin (Poudel et al., 2023). Distinct extracts obtained with different solvents do not have similar phytochemical profiles, and thus will exhibit different biological activity (Moomin et al., 2023). Apart from the influence of solvent polarity, the extraction yields also depend on the temperature and extraction time (Moomin et al., 2023).

3.1.3.3. DPPH assay

The overall results showed that the free radical scavenging activity of the green variety was higher compared to the other varieties, except for the ethanol extract. The lowest value was recorded in Kabuli variety of *T. chebula* extracted in water and ethanol (Table 4). For all the studied varieties, the best solvent was proved to be water since the extracts exhibited the greatest antiradical activity. Sheng et al. (2018) and Parveen et al. (2018) also investigated the DPPH value in various extracts of *T. chebula*, in different solvents like water, hexane, acetone, methanol, ethyl acetate, and ethanol, and reported 77–85% inhibition. Similarly, Shaikh et al. (2016) also studied the DPPH inhibition in water, ethanol, and acetone and obtained 80 to 85% inhibition of chebulic myrobalan plant (*T. chebula*). The main components responsible for the inhibition of free radicals through hydrogen atoms donation are phenolic compounds and flavonoids (Aryal et al., 2019). The differences between the extraction methods using different solvents could be attributed to the ability of each solvent to better solubilize certain components. For example, the acetone extract of *T. chebula* had high amounts of hydroxybenzoic acid derivatives, hydroxycinnamic acid derivatives, flavonols, aglycon, and glycosides (Hassan Bulbul et al., 2022). On the other hand, the ethanol extract is rich in total terpenoid content, while the aqueous extract contains mainly phenolic compounds and tannins (Hassan Bulbul et al., 2022).

3.1.3.4. FRAP assay

The current results showed that the green variety contains a higher value for the extracts in ethanol, acetone and hexane compared to other varieties, while the black sample had the best antioxidant activity in water (Table 4). The best solvent for the green sample was acetone (73.41 mmol/100 g), while for the black and Kabuli water was the most effective (113.4 mmol/100 g and 76.33 mmol/100 g respectively). Arya et al. (2013) and Dixit et al. (2013) showed that the FRAP value ranged from 93.97 to 109.6 mmol/100 g, respectively. *T. chebula* is rich in tannins like gallic acid, ellagic acid, chebulic acid, chebulinic acid, punicalagin, terflavin A, corilagin, galloyl glucose, tannic acid, while among the most abundant flavonoids, quercetin, catechin, and kaempferol can be mentioned (Hassan Bulbul et al., 2022). These compounds contribute to the antioxidant properties of Harad. The differences among varieties regarding the best solvent could be attributed to the differences in the phenolic profile of the samples, being known that each solvent has an affinity for distinct phenolics category (Hassan Bulbul et al., 2022).

3.2. Muffins characterization

3.2.1. Color analysis of *Terminalia chebula*-supplemented muffins

The results for color showed that the lightness L* of *T. chebula-supplemented* muffins ranged from 37.86 to 45.83 (Table 5). The highest lightness value (52.88) was detected in the control and the lowest value in T11 (10% Kabuli *T. chebula*). The redness value of muffins varied from 5.46 to 9.90. The T0 (control) contains the lowest value of 5.46, while T12 (15% Kabuli *T. chebula*) showed the highest color tonality 9.90. The yellowness value fluctuated from 17.21 to 27.65 in the samples with different addition levels of the three varieties of *T. chebula*. A maximum value of 27.65 was recorded in T8 (15% black *T. chebula*), and the lowest value of 17.21 in T11 (10% Kabuli).

The calculated chroma value of different formulations of *T. chebula* varied from 19.46 to 28.23. The highest C* value was recorded in T8 (15% black-supplemented muffins) - 28.23, while T11 (10% Kabuli *T. chebula*) showed the lowest value - 19.46. The h* value of *T. chebula*-supplemented muffins ranged from 78.25 to 62.13. The highest value was observed in T8 (15% black) - 78.25, and for T11 (10% Kabuli), the lowest value was obtained (62.13). Arifin et al. (2019) and Topkaya and Isik (2019) investigated the color tonality value in supplemented muffins with pumpkin puree and pomegranate peel at different concentrations and reported similar values. The color differences could be due to the intensification of Maillard reactions and/or to the pigments present like carotenoids and gallotannins in *T. chebula* (Saxena and Saxena, 2004). Other tannins that could have contributed to the color changes are gallic acid, ellagic acid, chebulic acid, chebulinic acid, punicalagin, terflavin A, corilagin, galloyl glucose, tannic acid (Hassan Bulbul et al., 2022). Color changes could be also due to the transformation of anthocyanins to other compounds during baking (De Oliveira et al., 2014).

3.2.2. Sensory textural parameters

Some textural parameters (softness, hardness, gumminess, and springiness) of *T. chebula*-supplemented muffins were determined and presented in Table 6. The results of softness and hardness showed significant variations ($p < 0.05$), the green-containing sample T1 exhibiting the highest value of 13.05 and 12.98 respectively, and the T12 Kabuli sample the lowest (8.66 and 9.99 respectively). Regarding the gumminess, T3 with green variety sample showed the greatest value, while in terms of springiness T5 with 5% black variety presented the best score.

The lowest values for gumminess and springiness were observed in T8 (15% black) (9.09 and 8.09). Topkaya and Isik (2019) and Jeong and Chung (2019) reported that the textural characteristics (springiness, hardness, softness, and gumminess) of pomegranate peel-supplemented muffins and legume-based muffins decreased with the increase of the powder concentration. The changes in the texture profile of muffins could be related to the fiber content of the ingredients added which could be responsible for disturbance of the gluten matrix. Generally, fruit fiber has a great affinity for water and the addition of fruit ingredients in muffins reduces the cake volume and causes a firmer, gummier, and less cohesive final product (Quiles

TABLE 5 Effect of different Harad varieties supplementation on the color tonality of muffins.

Treatment	L*	a*	b*	C*	h*
T_0	52.88 ± 1.96^{a}	5.46 ± 1.43^{b}	23.46 ± 0.61^{bcd}	24.10 ± 1.98^{d}	76.90 ± 1.91^{ab}
T_1	38.7 ± 0.16^{gh}	9.73 ± 0.75^{a}	20.49 ± 1.05^{ef}	22.67 ± 1.97^{def}	64.62 ± 1.88^{de}
T_2	39.52 ± 0.29^{fgh}	9.67 ± 0.81^{a}	20.98 ± 1.13^{def}	23.23 ± 1.83^{bcd}	64.73 ± 1.74^{de}
T_3	40.47 ± 0.36^{efg}	9.54 ± 0.85^{a}	21.88 ± 1.76^{cde}	23.85 ± 1.81^{bc}	66.44 ± 1.65^{cd}
T_4	41.44 ± 0.46^{de}	9.20 ± 0.96^{a}	23.12 ± 1.94^{bcd}	24.91 ± 1.43^{c}	68.10 ± 1.44^{c}
T_5	43.03 ± 1.41^{cd}	6.65 ± 1.23^{b}	24.76 ± 1.85^{b}	25.63 ± 1.6^{b}	74.97 ± 1.40^{b}
T_6	43.87 ± 0.11^{c}	5.99 ± 1.21^{b}	24.67 ± 1.71^{b}	25.37 ± 1.76^{b}	76.35 ± 1.46^{ab}
T_7	44.18 ± 0.54^{bc}	5.84 ± 1.20^{b}	24.28 ± 1.56^{bc}	24.77 ± 1.89^{c}	76.47 ± 1.49^{ab}
T_8	45.83 ± 0.50^{b}	5.75 ± 1.18^{b}	27.65 ± 1.41^{a}	28.23 ± 1.99^{a}	78.25 ± 1.58^{a}
T_9	42.42 ± 1.14^{cd}	8.85 ± 1.22^{a}	22.66 ± 1.72^{bcde}	24.31 ± 1.98^{d}	68.67 ± 1.88^{c}
T_{10}	41.32 ± 1.21^{def}	8.93 ± 0.84^{a}	18.99 ± 1.79^{fg}	20.97 ± 1.96^{bc}	66.82 ± 1.74^{cd}
T_{11}	37.86 ± 1.06^{h}	9.10 ± 0.44^{a}	17.21 ± 1.84^{g}	19.46 ± 1.88^{de}	62.13 ± 1.66^{e}
T_{12}	39.54 ± 0.05^{fgh}	9.90 ± 0.30^{a}	21.49 ± 1.87^{def}	23.66 ± 1.68^{bcd}	65.22 ± 1.56^{d}

T_0, Control (100% WF); T_1, 5.0% green Harad powder and 95% WF; T2, 7.5% green Harad powder and 92.5% WF; T3, 10% green Harad powder and 90% WF; T4, 15% green Harad powder and 85% WF; T5, 5% black Harad powder 95% WF; T6, 7.5% black Harad and 92.5% WF; T7, 10% black Harad powder and 90% WF; T8, 15% black Harad powder and 85% WF; T9, 5% Kabuli Harad powder and 95% WF; $T_1$0, 7.5% Kabuli Harad powder and 92.5% WF; $T_1$1, 10% Kabuli Harad powder and 90% WF; $T_1$2, 15% Kabuli Harad powder and 85% WF. The different letters on the same column show a significant difference according to Duncan's test ($p < 0.05$).

TABLE 6 Effect of different Harad varieties supplementation on the textural properties of muffins.

Treatment	Softness	Hardness	Gumminess	Springiness
T_0	14.09 ± 0.36^a	13.04 ± 0.43^a	10.05 ± 0.57^d	11.02 ± 0.47^{ab}
T_1	13.05 ± 0.41^b	12.98 ± 0.52^a	13.02 ± 0.47^a	11.05 ± 0.41^{ab}
T_2	12.02 ± 0.46^c	12.01 ± 0.48^b	12.03 ± 0.45^b	10.23 ± 0.64^{bc}
T_3	12.07 ± 0.39^c	12.16 ± 0.28^b	13.05 ± 0.42^a	10.02 ± 0.46^{cd}
T_4	8.04 ± 0.43^f	11.05 ± 0.41^c	12.05 ± 0.41^b	10.01 ± 0.48^{cd}
T_5	11.99 ± 0.50^c	12.08 ± 0.37^b	11.04 ± 0.43^c	11.12 ± 0.33^a
T_6	11.03 ± 0.72^d	11.10 ± 0.35^c	11.06 ± 0.40^c	10.06 ± 0.41^{cd}
T_7	10.33 ± 0.76^{de}	12.22 ± 0.68^b	11.05 ± 0.03^c	9.16 ± 0.76^e
T_8	10.17 ± 0.62^e	11.00 ± 0.49^c	9.09 ± 0.36^e	8.09 ± 0.64^f
T_9	12.04 ± 0.44^c	12.01 ± 0.48^b	12.05 ± 0.41^b	11.02 ± 0.47^{ab}
T_{10}	12.05 ± 0.42^c	11.97 ± 0.54^b	12.00 ± 0.49^b	11.05 ± 0.41^{ab}
T_{11}	10.28 ± 0.25^{de}	10.83 ± 0.28^c	11.02 ± 0.47^c	10.05 ± 0.42^{cd}
T_{12}	8.66 ± 0.57^f	9.99 ± 0.01^d	10.66 ± 0.57^{cd}	9.33 ± 0.28^{de}

T_0, Control (100% WF); T_1, 5.0% green Harad powder and 95% WF; T2, 7.5% green Harad powder and 92.5% WF; T3, 10% green Harad powder and 90% WF; T4, 15% green Harad powder and 85% WF; T5, 5% black Harad powder 95% WF; T6, 7.5% black Harad and 92.5% WF; T7, 10% black Harad powder and 90% WF; T8, 15% black Harad powder and 85% WF; T9, 5% Kabuli Harad powder and 95% WF; $T_1$0, 7.5% Kabuli Harad powder and 92.5% WF; $T_1$1, 10% Kabuli Harad powder and 90% WF; $T_1$2, 15% Kabuli Harad powder and 85% WF. The different letters on the same column show a significant difference according to Duncan's test ($p < 0.05$).

et al., 2018). Another major component of *T. chebula* that might have influenced the texture of the muffins is sugars. Sugar is responsible for disturbance in gluten matrix development and increases starch gelatinization and protein denaturation temperatures which led to the tenderizing of muffins texture (Quiles et al., 2018).

3.2.3. Sensory evaluation

The sensory profile of muffins indicated significant variations ($p < 0.05$) among the different amounts of *T. chebula* varieties supplementation. The data are presented in Table 7. The crust color was dark due to the Maillard reaction, and the results of the Kabuli and green varieties were close (13.28 and 13.07, respectively).

The black T8 sample showed the lowest score of crust color (8.02), while the highest value was recorded in the control (14.01) and in T10 (7.5% Kabuli *T. chebula*) (13.28). The crumb was a small portion of the muffins and represented the inner part just below the crust. The black T8 and green T4 showed close values for crumb color of 7.03 and 7.10, while the highest value was recorded in the control T0 (14.13). The highest score for crust appearance was in T0 (14.02), while T8 (15% black) showed the lowest score (7.03). The greatest value for crumb appearance was observed for T0 (control) (13.02), and the lowest for T4 (15% green *T. chebula*) (8.21).

The volume and aroma were important parameters in the sensory evaluation. The results of volume showed that among the supplemented muffins, the highest value was observed in green *T. chebula* containing sample T1 (12.07). In contrast, the lowest value was recorded in black T8 (8.02). The T0 (control) exhibited the highest value for volume and aroma (14.15 and 13.05, respectively). The worst score for aroma was obtained by T7 sample (6.09). The taste characteristics results showed that among the supplemented muffins, the highest score was obtained by black T6 sample (13.06). At the same time, the lowest value was recorded in green T4 sample (5.16). T0 (Control) showed the highest value in both parameters, taste and mouth feel (13.11 and 14.12), respectively. With respect to mouthfeel, the best score was obtained by control T0 (14.12), followed by the T1 sample (13.29), while the smallest score was exhibited by T12 (9.66).

The over-acceptability parameter revealed the significant impact of different *T. chebula* varieties, showing that the Kabuli-containing muffins obtained better scores compared to the black variety. The lowest value was observed for T8 (15% black) (7.03), while the highest value was obtained for T10 (7.5% Kabuli) (13.24). Shabeer et al. (2016) and Arya et al. (2013) conducted a study in which mango kernel, casein, and egg white were added to cookies to investigate the sensory profile. The authors reported significant variations in color, and a decrease in flavor and overall acceptability scores, changes that were related to the Maillard reactions and to the presence of high amounts of fiber, ash, and low protein level of the ingredient added. The taste, aroma, and consequently general acceptability of muffins supplemented with *T. chebula* could have been influenced by the presence of polyphenols. There are some phenolic compounds that give a bitter taste even in very small concentrations (De Oliveira et al., 2014). The sweet and bitter tastes are mutually suppressed in blends, while the astringency and bitterness can be considered negative features by the panelists (De Oliveira et al., 2014). Thus, the decrease in sensory scores of muffins could be attributed to the intake of phenolic compounds from *T. chebula.*

3.3. Relationships between characteristics and samples

3.3.1. Harad varieties characteristics

The relationships between the evaluated characteristics of Harad varieties (Figure 1) and supplemented muffins (Figure 2) were evaluated through Principal Component Analysis. For Harad varieties characteristics, the first principal component (PC1) explained 58.35% of data variation, while the second explained 41.63% of the total variance (Figure 1). Green and Kabuli varieties were associated with PC1, while the Black variety was associated with PC2. The contents of Ni, Na, Ca, Pb, K, Fiber, Ash, DPPH in all solvents, TPC and TFC in ethanol and acetone were associated with PC1, while Cd, Zn, protein, fat, TPC, FRAP, and TFC in hexane and

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TABLE 7 Effect of different Harad varieties supplementation on the sensory characteristics of muffins.

Treatment	Crust color	Crumb color	Crust appearance	Crumb appearance	Volume	Aroma	Taste	Mouth feel	Overall acceptability
T_0	14.01 ± 0.02^{a}	14.13 ± 0.69^{a}	14.02 ± 0.46^{a}	13.02 ± 0.46^{a}	14.15 ± 0.35^{a}	13.05 ± 0.41^{a}	13.11 ± 0.29^{a}	14.12 ± 0.32^{a}	13.16 ± 0.28^{a}
T_1	13.07 ± 0.39^{bc}	11.91 ± 0.60^{bc}	11.05 ± 0.41^{b}	12.03 ± 0.55^{ab}	12.07 ± 0.43^{b}	10.16 ± 0.29^{b}	11.02 ± 0.47^{c}	13.29 ± 0.60^{b}	12.49 ± 0.49^{ab}
T_2	12.02 ± 0.53^{d}	11.16 ± 0.75^{c}	10.16 ± 0.76^{c}	$11.05\ 0.58^{c}$	11.01 ± 0.51^{c}	9.02 ± 0.54^{c}	10.05 ± 0.58^{d}	13.06 ± 0.41^{bc}	13.03 ± 0.45^{a}
T_3	10.26 ± 0.55^{ef}	9.06 ± 0.42^{e}	9.026 ± 0.54^{d}	10.44 ± 0.52^{cd}	10.02 ± 0.53^{d}	7.13 ± 0.32^{d}	7.08 ± 0.37^{g}	11.97 ± 0.53^{e}	10.01 ± 0.47^{c}
T_4	8.13 ± 0.70^{h}	7.10 ± 0.36^{g}	8.16 ± 0.29^{e}	8.21 ± 0.39^{g}	9.08 ± 0.21^{e}	10.13 ± 0.32^{b}	5.16 ± 0.76^{h}	10.09 ± 0.36^{g}	7.05 ± 0.57^{d}
T_5	12.02 ± 0.54^{d}	12.10 ± 0.65^{b}	11.23 ± 0.58^{b}	12.15 ± 0.36^{ab}	11.16 ± 0.74^{c}	9.03 ± 0.45^{c}	12.03 ± 0.45^{b}	12.25 ± 0.66^{cde}	13.02 ± 0.46^{a}
T_6	11.01 ± 0.43^{e}	10.05 ± 0.41^{d}	9.09 ± 0.36^{d}	10.06 ± 0.57^{de}	11.31 ± 0.55^{b}	7.08 ± 0.38^{d}	13.06 ± 0.40^{a}	12.07 ± 0.39^{de}	12.56 ± 0.27^{ab}
T_7	9.34 ± 0.59^{g}	8.056 ± 0.58^{f}	8.09 ± 0.36^{e}	9.07 ± 0.85^{fg}	9.05 ± 0.41^{e}	6.09 ± 0.64^{e}	8.99 ± 0.51^{e}	10.02 ± 0.47^{g}	10.12 ± 0.03^{c}
T_8	8.02 ± 0.53^{h}	7.03 ± 0.54^{g}	7.03 ± 0.54^{f}	8.28 ± 0.95^{g}	8.02 ± 0.53^{f}	9.16 ± 0.29^{c}	8.05 ± 0.58^{f}	9.72 ± 0.86^{g}	7.03 ± 0.55^{d}
T_9	12.45 ± 0.42^{cd}	11.05 ± 0.41^{c}	11.13 ± 0.32^{b}	11.09 ± 0.36^{c}	11.15 ± 0.29^{c}	10.02 ± 0.47^{b}	11.31 ± 1.06^{bc}	12.05 ± 0.42^{de}	13.04 ± 0.43^{a}
T_{10}	13.28 ± 0.61^{ab}	12.13 ± 0.32^{b}	11.16 ± 0.29^{b}	12.30 ± 0.59^{ab}	11.05 ± 0.41^{c}	10.13 ± 0.33^{b}	12.08 ± 0.37^{b}	12.83 ± 0.28^{bcd}	13.24 ± 0.65^{a}
T_{11}	9.77 ± 0.36^{fg}	10.02 ± 0.46^{d}	10.09 ± 0.64^{c}	9.50 ± 0.53^{ef}	10.02 ± 0.46^{d}	7.29 ± 0.51^{d}	11.97 ± 0.54^{b}	11.12 ± 0.33^{f}	12.16 ± 0.75^{b}
T_{12}	9.13 ± 0.32^{g}	8.26 ± 0.45^{ef}	8.56 ± 0.40^{de}	8.59 ± 0.16^{fg}	9.63 ± 0.22^{de}	9.16 ± 0.29^{c}	7.31 ± 0.35^{fg}	9.66 ± 0.57^{g}	7.59 ± 0.24^{d}

T_0, Control (100% WF); T_1, 5.0% green Harad powder and 95% WF; T2, 7.5% green Harad powder and 92.5% WF; T3, 10% green Harad powder and 90% WF; T4, 15% green Harad powder and 85% WF; T5, 5% black Harad powder 95% WF; T6, 7.5% black Harad and 92.5% WF; T7, 10% black Harad powder and 90% WF; T8, 15% black Harad powder and 85% WF; T9, 5% Kabuli Harad powder and 95% WF; $T_1$0, 7.5% Kabuli Harad powder and 92.5% WF; $T_1$1, 10% Kabuli Harad powder and 90% WF; $T_1$2, 15% Kabuli Harad powder and 85% WF. The different letters on the same column show a significant difference according to Duncan's test ($p < 0.05$).

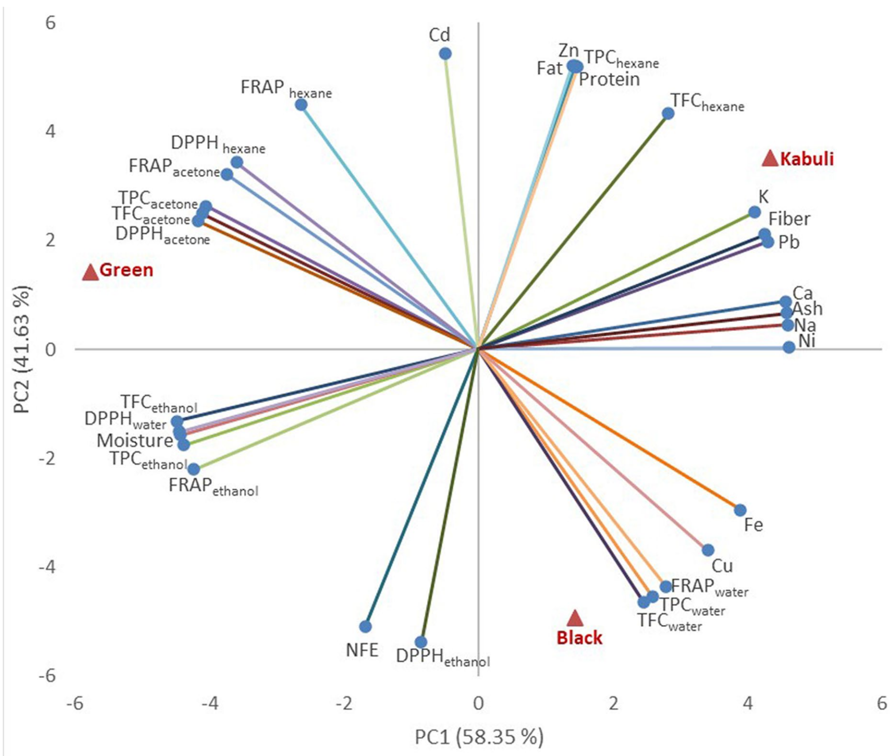

FIGURE 1
Principal Component Analysis bi-plot for the Harad varieties (red triangles) and the evaluated variables (blue bullets): NFE-nitrogen free extract, TPC-total phenolic content, TFC-total flavonoid content, FRAP- ferric reducing antioxidant power assay, DPPH- 2,2-diphenylpicrylhydrazyl assay.

water, NFE and DPPH in ethanol were associated to PC2. TPC, TFC, and FRAP in ethanol are opposed to K, fiber, and Pb content, while TPC, TFC, FRAP, and DPPH in acetone are placed in opposition to Fe, Cu, FRAP, opposed to Fe, Cu, FRAP, respectively TPC and TFC in water. The Zn, TPC in hexane, protein, and fat variables oppose DPPH ethanol and NFE (Figure 1). Moisture was positively correlated with TPC and TFC in ethanol ($p < 0.05$, $0.59 < r < 0.91$), while ash content was strongly correlated with Ca and Na ($p < 0.05$, r = 0.99). Some strong correlations were observed between some TPC and TFC and DPPH and FRAP variables ($p < 0.05$). Fe content was negatively correlated with TPC acetone ($p < 0.05$, r = 0.99). At the same time, Zn, fat, and protein were strongly positively correlated with TPC hexane ($p < 0.05$, r = 0.99) and negatively with DPPH ethanol ($p < 0.05$, $r = 0.99$). The literature also reported significant correlations between the TPC and TFC with the antiradical activity of plants (Aryal et al., 2019).

3.3.2. Muffins characteristics

Data variability regarding muffin samples and their characteristics was explained 54.62% by PC1 and 26.11% by PC2. The sensory and texture properties, except gumminess, were associated with PC1, while the color parameters and gumminess were associated with PC2. The muffins containing the black Harad variety were placed in the upper part of the graphic, while those containing green and Kabuli Harad were positioned in the lower part of the plot (Figure 2).

The control sample was differentiated from the others, being placed in the right positive quadrant. Softness, hardness, and springiness were positively correlated ($p < 0.05$, $0.61 < r < 0.91$) with the sensory characteristics of crust and crumb (color and appearance), volume mouth feel, and overall acceptability. Softness and springiness were positively correlated with taste ($p < 0.05$, $r = 0.59$, and $r = 0.69$, respectively). Positive correlations were also found between crust or crumb color or appearance and overall acceptability, mouth feels, taste, and volume ($p < 0.05$, $0.72 < r < 0.94$).

4. Conclusion

It is concluded that the Kabuli Harad and green varieties have higher proximate and mineral content than the black variety. The black Harad contained a higher antioxidant potential. The color tonality of the supplemented muffins showed that the black variety had the highest value compared to the other varieties at different levels. In the case of product development, the 7.5% level for each of the three varieties (Kabuli, black, and green) exhibited good acceptability of muffins. Considering these results, the present study has confirmed the feasibility of supplementing white flour with different *Terminalia chebula* varieties to develop functional muffins. Further investigation of the effects of *Terminalia chebula* on the rheological properties of the batter, final product microstructure, and consumption benefits is needed.

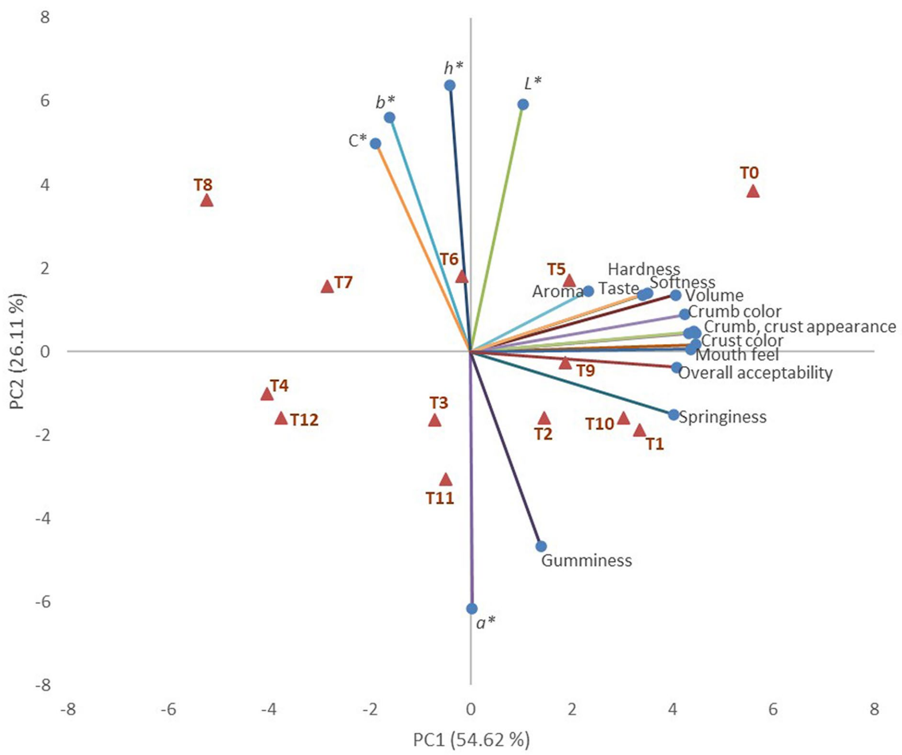

FIGURE 2
Principal Component Analysis bi-plot for the enriched muffins (red triangles) and the studied variables (blue bullets): T0-Control (100% WF), T1-5.0% green Harad powder and 95% WF, T2-7.5% green Harad powder and 92.5% WF, T3-10% green Harad powder and 90% WF, T4-15% green Harad powder and 85% WF, T5-5% black Harad powder 95% WF, T6-7.5% black Harad and 92.5% WF, T7:10% black Harad powder and 90% WF, T8:15% black Harad powder and 85% WF, T9:5% Kabuli Harad powder and 95% WF, T10-7.5% Kabuli Harad powder and 92.5% WF, T11-10% Kabuli Harad powder and 90% WF, T12-15% Kabuli Harad powder and 85% WF.

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

MTS and EK: conceptualization and investigation. WK: methodology, software, and data curation. WK and MZK: validation. SRS and MAR: formal analysis. IC: resources. EK and MU-I: writing-original draft preparation. CM, MU-I, and IC: writing-review and editing. MI-L, AB, and CM: visualization. SM: supervision, project administration, and funding acquisition. 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.

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

Publisher's note

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Assessing the impact of drum drying on the nutritional properties of pineapple pomace-fortified crispy mushroom sheets

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The processing of 'Phulae' pineapple fruit for juice generates a significant by-product called pineapple pomace (PAP) that has attracted interest from the functional snack industry. Pineapple pomace (PAP) is rich source of dietary fiber and antioxidants, which make it suitable for incorporating into mushroom products that are rich in high protein content. To investigate the impact of drum drying parameters on the physicochemical properties of PAP, response surface methodology (RSM) was employed for optimization of processing parameters such as steaming temperature (130 to 170°C) and rotation speed (1 to 3 rpm). The aim was to determine the optimal conditions for achieving the highest yield of diphenylpicrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and soluble dietary fiber (SDF), which are important indicators of antioxidant activity and dietary fiber content. These optimal conditions would be used in the production of crispy *Pleurotus sajor-caju* (PS-PAP) and *Lentinus squarrosulus* (LS-PAP) sheets both of which were supplemented with PAP. The response of PS-PAP and LS-PAP to the independent variables (steaming temperature and rotation speed) was adequately described by a second-order polynomial equation. The equations demonstrated a high degree of fit with respect to DPPH (R^2 = 0.9693 for PS-PAP sheet and R^2 = 0.9966 for LS-PAP sheet), FRAP (R^2 = 0.9908 for PS-PAP sheet and R^2 = 0.9877 for LS-PAP sheet), and SDF (R^2 = 0.9689 for PS-PAP sheet and R^2 = 0.9598 for LS-PAP sheet). Moreover, the experimental values of the dependent variables closely matched the predicted values, indicating the reliability of the generated models. It was evident that both steaming temperature and rotation speed significantly influenced DPPH, FRAP, and SDF contents and the optimized conditions could be employed for the production of functional crispy mushroom sheets. In conclusion, the study's novelty lies in the optimization of PAP drum drying parameters for the production of functional crispy mushroom sheets. The research creates opportunities for sustainable and nutritious products, and future perspectives could include sensory evaluation, nutritional analysis, shelf-life studies, market potential and environmental impact assessment. Overall, this research contributes to the development of healthier and eco-friendly food options.

KEYWORDS

pineapple pomace, mushroom, drum drying, response surface optimization, bioactive properties, functional snacks

Introduction

Over the past few years, more and more people have become aware of the links between health and diet, and there has been a trend toward healthier eating. The utilization agricultural waste byproducts such as straw, leaves, peel or pomace from fruits and vegetables has led to the development of new functional food products that cater to various consumer groups including those who consciously choose products with health-promoting properties. Nowadays there is growing awareness of the consequences for health that necessitates the consumption of plant-based protein from cereals, legumes, oil seeds, nuts, pseudocereals, tubers, fruits, vegetables, and mushrooms to reduce the consumption of animal meat (Langyan et al., 2021). Although legumes are a promising protein source, some consumers have pea protein allergies (Hertzler et al., 2020). As a result, there has been an increasing popularity in using mushrooms as the primary raw material for developing alternative protein products, catering to a broader range of consumers. Thailand, in particular, produces a wide variety of edible mushrooms, providing opportunities to diversify product options. It has been reported that oyster mushroom (*Pleurotus sajor-caju*) (PS) and white log (*Lentinus squarrosulus* Mont.) (LS) contains protein content of 50.29 ± 4.17% and 40.34 ± 3.23% by dry weight, respectively (Srikram and Supapvanich, 2016). These mushrooms can serve as essential ingredients in the production of high-protein health food products. Furthermore, their cost-effectiveness and widespread cultivation across the country ensure their year-round availability.

'Phulae' pineapple (*Ananas comosus* L. Merr.) holds significant geographical indications as an agricultural product of Chiang Rai Province, Thailand. It is highly nutritious, containing a wealth of vitamins, fibers, and minerals (Kongsuwan et al., 2009). One popular method of processing this variety is juice extraction, which yields valuable by-products such as peel, crown, core, and pomace that together constitute approximately 45–55% of the total pineapple weight (Banerjee et al., 2018). Among these by-products, pineapple pomace (PAP) stands out due to its immense potential for conversion into economically viable functional foods. PAP is a rich source of insoluble dietary fiber, compared to soluble dietary fiber (Jose et al., 2022). Insoluble dietary fiber (IDF) supports a healthy digestive tract, while soluble dietary fiber (SDF) aids in lowering LDL cholesterol and blood sugar levels (Liu et al., 2022). By incorporating PAP as an ingredient, the issue of low dietary fiber content in regular snacks can be addressed, thereby enhancing their functional properties and promoting a healthy digestive system. Moreover, dietary fiber possesses other beneficial properties such as water absorption capacity, oil holding capacity, and swelling capacity that are advantageous during the drum drying process (Zhang et al., 2020).

Twin drum drying is an alternative drying method that offers a fast dehydration rate, similar to refractance window and spray drying technologies, each of which has limitations and advantages (Caparino et al., 2012; Karthik et al., 2017). Twin drum drying is considered the easiest and most economical method of food processing in which a food slurry is spread onto a surface then rapidly heated by steaming between the drums to ensure crispy texture of food (Hidayat et al., 2021). The drums rotate to generate heat, aid in feeding the slurry, and discharge the dehydrated product using a scraper blade. The steaming temperature and holding time influence the degree of gelatinization, and drying rate (Taşkın and Savlak, 2022). Drum drying is commonly used for producing breakfast cereals, bakery foods, beverages, and dairy products, as the resulting dried product typically takes the form of flakes or powder (Wiriyawattana et al., 2018; Taşkın and Savlak, 2022). However, achieving a product with sheet-like characteristics through drum drying presented a significant challenge in this study. Previous research focused on developing a crispy 'Phulae' pineapple product from PAP using a drum dryer. The results of this preliminary study using a drying temperature of 150°C and a rotation rate of 2 rpm resulted in a product containing 11.1% dietary fiber and 4.2% protein. The product can be consumed as a functional snack with high dietary fiber content (not less than 6 g per 100 g of product, as listed in Attachment No. 4 of the Notification of the Ministry of Public Health No. 182, B.E. 2,541). However, the product did not consistently achieve a sheet-like texture, necessitating the addition of a binding or stabilizing agent, such as hydrocolloids (Li and Nie, 2016). The addition of flour and fortification with hydrocolloids tends to enhance thixotropic behavior of mushroom-based PAP sheets (Martínez et al., 2015).

This study aimed to develop a high-protein, crispy mushroom product enriched with healthy dietary fiber by utilizing the twin drum drying process that produces sheet-like characteristics. This product would serve as a plant-based protein rich functional product. The objective of this study was to investigate the influence of steaming temperature (130°C, 150°C, 170°C) and rotation rate (1, 2 and 3 rpm) on the functional properties of the mushroom sheets. The experimental runs were designed using the Box–Behnken design (BBD), which enables the creation of sequential designs, calculation of quadratic model parameters, and detection of the model's lack of fit (Jafari et al., 2023). Subsequently, optimal drying conditions were determined through the use of response surface methodology (RSM) and were used to develop prototype products consisting of crispy mushroom sheets fortified with PAP. The products were evaluated for the proximate composition, physicochemical properties and dietary fiber content.

Materials and methods

Procurement of raw materials

Pineapple pomace (PAP) from 'Phulae' pineapples was obtained from a pineapple juice factory in Chiang Rai. The PAP was washed three times with deionized water in a 1:3 ratio of pomace to water to reduce acidity and provide a less sour taste. After reducing the acidity, the water content was reduced using hydraulic press. The processed PAP was stored at −20°C until use to prevent fermentation by lactic acid bacteria. The oyster (*Pleurotus sajor-caju*) (PS) and white log (*Lentinus squarrosulus* Mont.) (LS) mushrooms were obtained from the Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai. Both PAP and mushroom samples were stored in a freezer at −20°C prior to the sample preparation and analysis.

Preparation of mushroom sheets fortified with pineapple pomace

The mixture was created by separately mixing PS (85% dry weight basis, db) and LS (85% db) with PAP (12% db), tapioca flour

(1% db), sugar (1% db), and xanthan gum (0.17% db) and blended with water in a ratio of 1:1 in preparation for the drum drying process. The slurry samples were then allowed to dry in a twin drum dryer. To obtain dried samples, the temperature was controlled at 130°C, 150°C, and 170°C, while the rotation speed was fixed at either 1, 2, and 3 rpm. Thin and crispy sheets of PAP, PS or LS fortified with PAP were obtained as the finished product after the twin drum drying process and were sealed in a container with silica oxide and nitrogen gas filling the headspace to remove any residual atmospheric air.

Experimental design and method validation for the preparation of mushroom sheets supplemented with pineapple pomace

The Box–Behnken design (BBD) for response surface methodology was employed to optimize the levels of steaming temperature and rotation speed of drum drying and their impact on the antioxidant and dietary fiber responses. Two factors, steaming temperature (X1: 130°C to 150°C) and rotation speed (X2: 1 to 3 rpm), were coded into five levels (−1.414, −1, 0, 1, 1.414), representing the lowest to highest values, respectively (Table 1). A total of thirteen experiments were conducted based on the natural variable design. To validate the model generated by the software under the optimal conditions, an experiment was performed, and the experimental values were compared with the predicted values. The experiment was conducted in duplicate (n = 2), and the composition analysis was carried out in triplicate (n = 3). The response surface was analyzed to validate the regression coefficients, determine the statistical significance of the model, and fit the mathematical models to optimize the dependent variable. The following quadratic polynomial equation [1] was used to fit the experimental data and investigate the response function (Y):

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad (1)$$

In the equation, Y represents the dependent variable, β0 is a constant coefficient, and β1, β2, β11, β22, and β12, represent the linear, squared, and interaction coefficients, respectively. Non-significant coefficients were removed from the equation during the modeling process. A three-dimensional model graph was used to interpret the relevance of the factors and the responses. The variables of steaming temperature and rotation speed was established for numerical optimization. The superimposed contour graphs of significant (p < 0.05) response variables illustrated the optimal conditions and predicted response values.

Physicochemical properties of pineapple pomace

Various physicochemical parameters of pineapple pomace were measured including water activity (a_w), pH, titratable acidity (TA % citric acid), antioxidant potential (DPPH and FRAP assays) and insoluble (IDF) and soluble dietary fiber (SDF) (McCleary et al., 2013). The aw of the PAP sample was measured in triplicate at 25°C using a METER Aqualab 4TE Benchtop instrument. To prepare a PAP sample for pH measurement, the suspension was created by blending 10 g of PAP with 100 mL deionized water. The suspended sample was then centrifuged, and the supernatant collected for pH measurement using a pH meter (Model pH 700, Benchtop pH Meter). The 40 mL aliquot was titrated with 0.1 N NaOH to reach a pH of 6.5, and then the titratable acidity was expressed as grams of citric acid per 100 g of the sample.

The analysis of total dietary fiber was performed on dried PAP and mushroom sheets, both with and without PAP, were analyzed following the method of Bird et al. (2016) with some modifications. Briefly, 1 g of the samples in triplicate was subjected to sequential enzymatic digestion using heat-stable α-amylase, protease, and amyloglucosidase. The insoluble dietary fiber (IDF) was filtered, and then the residue was washed with warm distilled water. The filtrate and water washings were precipitated with 4 volumes of 95% ethanol for the determination of soluble dietary fiber (SDF). The precipitate was then filtered and dried. Both the SDF and IDF residues were adjusted for protein, ash, and blank values in order to calculate the final SDF and IDF contents. The SDF was precipitated with ethanol, and the residue was subsequently filtered, dried, and weighed. The total dietary fiber (TDF) content was adjusted for protein and ash content.

Antioxidant characteristics of crispy mushroom sheets supplemented with pineapple pomace

The DPPH method was used to determine radical scavenging activity in both dried PAP and mushroom sheets with and without PAP as described by Philip (2004). Briefly, the sample was extracted with deionized water at 50°C for 30 min in a sonication bath. The ratio of the sample to water was 1:5. The extract was then centrifuged at 10,000 rpm for 5 min. The supernatant was collected and filtered using a nylon filter membrane with a pore size of 0.45 μm. Then, 50 μL of the filtered sample was added to 1950 μL of methanolic DPPH (60 μM of 2,2′-diphenyl-1-picryl-hydrazyl in methanol) and vortexed for 30 s. The mixture was kept in a dark place at room temperature. After 30 min, the mixture was placed in a spectrophotometer to determine the absorbance at 517 nm against the blank. The DPPH scavenging

TABLE 1 Independent variables and levels in the Box–Behnken design (BBD).

Independent variables	Symbol	Coded values				
		−1.414	−1	0	1	1.414
Steam temperature (°C)	X_1	121.72	130.00	150.00	170.00	178.28
Rotation speed of drum (rpm)	X_2	0.59	1.00	2.00	3.00	3.41

activity was expressed as the percentage of scavenging using the following equation [2].

$$\text{DPPH scavenging activity (\%)} = 1\text{- Ac/Ad} \quad (2)$$

Where Ac is the absorbance of solution, Ad is the absorbance of DPPH solution.

The sample was extracted for ferric reducing antioxidant power (FRAP) analysis using the same method of sample preparation employed for DPPH scavenging activity. Ascorbic acid was used as a working standard to generate a calibration curve. Both the standard and sample were pipetted into test tubes, with 1 mL in each tube. The FRAP reagent was prepared by adding 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate and vortexed to mix well. The mixture was incubated in a thermostatic bath at 50°C for 30 min. Then, 2.5 mL of 10% trichloroacetic acid was added. The mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride were added. It was filtered through a 0.45 μm nylon membrane. The absorbance was measured at 700 nm against the blank. The FRAP was expressed as the reducing antioxidant power in the unit of μmol ascorbic acid/100 g wet basis (Zhong and Shahidi, 2015).

Microbiological quality evaluation of mushroom sheets with pineapple pomace

The determination of total plate counts (TPC), counts of yeasts and molds (YM), and counts of *Clostridium perfringens* followed the FDA BAM method (U.S. Food and Drug Administration, 2001) and was reported as CFU/g. *Bacillus cereus* counts were reported in the unit of CFU/g, while *Escherichia coli* counts were reported as MPN/g (U.S. Food and Drug Administration, 2020). *Staphylococcus aureus* was determined according to the FDA BAM (U.S. Food and Drug Administration, 2016) and reported in the unit of CFU/g. The determination of *Salmonella* spp. was carried out on a 25 g sample of the product, following the method of the International Organization for Standardization (2020). The report represented *Salmonella* spp. in 25 g of the product.

Statistical analysis

The data were statistically analyzed using Design-Expert Version 6.0.10 software. The completely randomized design was employed for the experimental design. The mean values of the three replicates of the response results were used for analysis. The R^2 values were determined to assess the fitness of the developed model, and the significance of the model was examined using analysis of variance (ANOVA) at ($p < 0.05$).

Results and discussion

Optimization of steaming temperature and rotation speed of a twin drum dryer on the antioxidant and dietary fiber content of pineapple pomace fortified in crispy mushroom sheets.

A Box–Behnken design (BBD) using two independent variables (*viz* steam temperature (X1) and rotation speed of the drum (X2)) was employed in the optimization of treatment conditions using response surface methodology (RSM). The coded values used for these variables were − 1.414, −1, 0, 1, and 1.414. The steam temperature (X1) varied between 121.72°C to 178.28°C, corresponding to the coded values of −1.414, −1, 0, 1, and 1.414, respectively. The rotation speeds of the drum (X2) ranged from 0.59 rpm to 3.41 rpm and the speeds were again assigned the coded values of −1.414, −1, 0, 1, and 1.414 (Table 1). The results of the optimization of twin drum drying of the PAP, PS-PAP and LS-PAP sheets using RSM-BBD are presented in Table 2. The table includes the experimental and predicted values for various response variables, such as DPPH scavenging activities, FRAP, and SDF contents at different combinations of steaming temperature and drum speeds. The DPPH scavenging activity ranged from 50.94 to 76.65% for the different experimental runs. The highest DPPH scavenging activity was observed at a steaming temperature of 150°C and a drum speed of 2 rpm and was predicted to be 74.66%. Similarly, the FRAP ranged from 230.45 μmol ascorbic acid/100 g to 277.89 μmol ascorbic acid/100 g. The highest FRAP was also observed at a steaming temperature of 150°C and a drum speed of 2 rpm and was predicted to be 270.75 μmol ascorbic acid/100 g. This suggests that the optimized drying conditions led to a high reducing power of the dried samples, which is indicative of its antioxidant capacity. The SDF content ranged from 1.26 g/100 g to 1.56 g/100 g. The highest SDF content was observed at a steaming temperature of 178.28°C and a drum speed of 2 rpm and was predicted to be 1.27 g/100 g.

The response surface methodology and the experimental data allowed for construction of a predictive model for the drying process. The predicted values obtained from the model were found to be in good agreement with the experimental values, indicating the reliability of the model in optimizing the drying process. The optimization results suggest that a steaming temperature of around 150°C and a drum rotation speed of 2 rpm are optimal for achieving high DPPH scavenging activity, FRAP, and SDF contents in the dried samples.

Table 3 presents the regression coefficients of the predicted second-order polynomial models for the antioxidant properties and dietary fiber content. The models were developed using the Box–Behnken design and the responses of the dependent variables to twin drum drying. For the antioxidant property, DPPH, the regression equation for the PAP sheet samples is given by:

$$\text{DPPH} = -566.95 - 0.03X_1^2 - 1.71X_2^2$$

The effects of steaming temperature and rotation speed on DPPH in the PAP sheets were found to be significant ($p < 0.05$) when fitted with a second-order quadratic linear effect (X_1^2, X_2^2) with no interaction occurring between the effects of steaming temperature and rotation speed. According to the equation, temperature has a greater impact on the response value compared to time. For PS-PAP sheets, a first-order quadratic linear effect (X_1) and a second-order quadratic linear effect (X_1^2, X_2^2) with no interaction term was found to be significant ($p < 0.05$). In the case of LS-PAP sheets, the effect of steaming temperature was more significant than rotation speed. Both steaming temperature and rotation speed were found to affect DPPH scavenging activity, with rotation speed having a greater impact than temperature. The regression coefficients (R^2) for PS-PAP and LS-PAP

TABLE 2 Optimization of twin drum drying: steaming temperature and rotation speed using response surface methodology.

Std. Order	Drying parameter		Response																	
	Steaming Temp. (° C)	Rotation speed of drum (rpm)	DPPH scavenging activity (%)						FRAP μmol ascorbic acid/ 100 g						SDF (g/ 100 g)					
			PAP sheet	PS-PAP sheet		LS-PAP sheet			PAP sheet	PS-PAP sheet		LS-PAP sheet			PAP sheet		PS-PAP sheet		LS-PAP sheet	
			Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred
1	130	1	63.05	62.51	45.53	44.80	46.33	45.14	212.73	212.07	145.84	144.92	148.28	142.49	1.42	1.43	5.81	5.73	6.12	6.25
2	170	1	62.15	61.47	52.14	52.01	53.93	53.20	239.92	241.15	191.14	193.74	209.99	208.25	1.53	1.56	6.17	6.17	6.49	6.79
3	130	3	61.31	62.66	52.18	52.10	53.36	52.86	235.12	235.62	158.66	161.57	171.86	174.42	1.47	1.49	5.88	5.84	6.19	6.44
4	170	3	58.73	59.94	57.25	57.77	57.37	57.34	247.33	249.72	174.78	181.21	180.26	186.87	1.56	1.58	6.26	6.28	6.59	6.98
5	121.72	2	53.79	53.37	35.76	36.27	35.23	36.18	197.23	197.70	119.96	119.69	122.56	125.02	1.53	1.56	6.13	6.17	6.45	6.79
6	178.28	2	50.94	50.71	45.73	45.38	44.75	45.04	230.45	228.24	173.36	168.11	183.58	180.32	1.26	1.27	5.03	5.00	5.29	5.54
7	150	0.59	70.73	71.74	57.35	57.89	58.35	59.46	245.01	244.96	195.41	195.36	194.11	199.62	1.55	1.56	6.07	6.17	6.39	6.79
8	150	3.41	72.42	70.76	67.51	67.13	67.73	67.86	269.35	267.66	203.75	198.28	213.38	207.08	1.27	1.28	5.00	5.08	5.26	5.58
9	150	2	74.87	74.66	69.71	68.09	69.75	69.39	265.79	270.75	227.37	226.32	263.03	254.24	1.53	1.54	6.05	6.06	6.37	6.74
10	150	2	76.65	74.66	68.93	68.09	69.13	69.39	269.03	270.75	229.44	226.32	256.09	254.24	1.55	1.56	6.25	6.17	6.58	6.79
11	150	2	73.83	74.66	67.95	68.09	68.72	69.39	277.89	270.75	226.50	226.32	252.63	254.24	1.56	1.56	6.20	6.17	6.53	6.79
12	150	2	73.81	74.66	71.35	68.09	69.35	69.39	275.73	270.75	225.44	226.32	248.27	254.24	1.50	1.51	5.87	5.96	6.18	6.50
13	150	2	74.12	74.66	62.54	68.09	69.97	69.39	265.33	270.75	222.87	226.32	251.17	254.24	1.45	1.45	5.74	5.75	6.04	6.38

PAP sheet, Pineapple pomace sheet; PS-PAP, Oyster mushroom (Pleurotus sajor-caju) (PS) fortified with PAP sheet; LS-PAP, white log (Lentinus squarrosulus Mont.) (LS) fortified PAP sheet.

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TABLE 3 Regression coefficient of the predicted second-order polynomial models (BBD) for antioxidant properties and dietary fiber content.

Sample	Regression coefficient	Polynomial equation (actual coefficient)	R^2	R^2 (Adjusted)	R^2 (Predicted)	ρ value	Lack of fit
PAP sheet	DPPH	$-566.95 - 0.03X_1^2 - 1.71X_2^2$	0.9849	0.9742	0.9276	< 0.0001	0.2744
	FRAP	$-1536.45 + 22.58X_1 + 65X_2 - 0.07X_1^2 - 7.22X_2^2$	0.9803	0.9662	0.9575	< 0.0001	0.9183
	IDF	-	0.9145	0.8534	0.4175	0.0013	0.0053
	SDF	$-6.34 + 0.09X_1 + 0.61X_2 - 0.003X_1X_2 - 0.0003X_1^2 - 0.025X_2^2$	0.9568	0.9260	0.7380	0.0001	0.0623
PS-PAP sheet	DPPH	$-746.13 + 10.42X_1 + 17.30X_2 - 0.03X_1^2 - 2.79X_2^2$	0.9693	0.9475	0.9460	< 0.0001	0.9836
	FRAP	$-2390.65 + 32.49X_1 - 0.36X_1X_2 - 0.1X_1^2 - 14.75X_2^2$	0.9908	0.9843	0.9435	< 0.0001	0.0509
	IDF	-	0.9162	0.8564	0.4250	0.0012	0.0036
	SDF	$-18.63 + 0.29X_1 + 1.73X_2 - 0.008X_1X_2 - 0.0008X_1^2 - 0.087X_2^2$	0.9689	0.9467	0.8236	< 0.0001	0.1140
LS-PAP sheet	DPPH	$-794.35 + 11.04X_1 + 21.15X_2 - 0.0.4X_1^2 - 2.86X_2^2$	0.9966	0.9942	0.9793	< 0.0001	0.0577
	FRAP	$-3056.12 + 40.40X_1 - 0.67X_1X_2 - 0.13X_1^2 - 25.45X_2^2$	0.9877	0.9789	0.9416	< 0.0001	0.2862
	IDF	-	0.9186	0.8605	0.4441	0.0011	0.0047
	SDF	$-21.08 + 0.33X_1 + 1.66X_2 - 0.007X_1X_2 - 0.001X_1^2 - 0.088X_2^2$	0.9598	0.9311	0.7533	< 0.0001	0.0537

PAP sheet, Pineapple pomace sheet; PS-PAP, Oyster mushroom (Pleurotus sajor-caju) (PS) fortified with PAP sheet; LS-PAP, white log (Lentinus squarrosulus Mont.) (LS) fortified PAP sheet.

sheets were 0.9693 and 0.9966, respectively, indicating a good fit. The predicted R^2 and adjusted R^2 values were in reasonably close agreement.

For the antioxidant property, FRAP, in the PAP sheets, the regression equation is:

$$FRAP = -1536.45 + 22.58X_1 + 65X_2 - 0.07X_1^2 - 7.22X_2^2$$

The R^2 value for this model is 0.9803, indicating that 98.03% of the variability in FRAP can be explained by the independent variables. The adjusted R^2 value is 0.9662, and the predicted R^2 value is 0.9575, indicating a good fit and predictive capability of the model. The regression (value of p) value is less than 0.0001, suggesting a significant relationship. The lack of fit is 0.9183, indicating a satisfactory fit of the model. Steaming temperature and rotation speed significantly affected the FRAP with a first-order quadratic linear effect (X_1, X_2) and a second-order quadratic effect (X_1^2, X_2^2), with no interaction occurring between steaming temperature and rotation speed. The interaction *p*-values for PS-PAP and LS-PAP sheets were 0.0135 and 0.0048, respectively, indicating significance. The R^2 values for PAP, PS-PAP, and LS-PAP sheets were 0.9803, 0.9908, and 0.9877, respectively, showing good predictability of the models.

Regarding dietary fiber, the regression equation for IDF was not included in the polynomial prediction due to a significant lack-of-fit ($\rho > 0.05$). Only SDF was fitted to the regression model. The SDF response of the PAP sheet showed a significant effect of steaming temperature and rotation speed. For PAP, PS-PAP, and LS-PAP sheets, all terms of the polynomial prediction equations were found to be significant for PAP, PS-PAP, and LS-PAP sheets. The significant polynomial terms include the first-order quadratic linear effects (X_1, X_2), second-order quadratic effects (X_1^2, X_2^2) and an interaction between steaming temperature and rotation speed (X_1X_2). The R^2 values for PAP, PS-PAP, and LS-PAP sheets were 0.9568, 0.9689, and 0.9598, respectively, indicating good predictability. Dong et al. (2019) reported that thermal processing can affect the structural and functional properties of SDF whole grain oats, leading to an increase in molecular weight, particle size, and molecular aggregation.

Fitting the model for optimizing the antioxidant and dietary fiber contents of pineapple pomace fortified with crispy mushroom sheets

Figure 1 shows the surface response with contours of the quadratic effects of steaming temperature and drum speed. The three-dimensional response surfaces for DPPH, FRAP, and SDF are presented individually for PAP, PS-PAP, and LS-PAP sheets. The contours' internal center helps visualize the impact of steaming temperature and rotation speed on the optimal conditions that yield the maximum response in the dependent variables. A second-order polynomial model was used to predict the response of the dependent variables, as shown in Table 3. The experimental values were then compared to the predicted values, revealing a close agreement between the two. This indicates that the model is reasonable for prediction. The twin drum drying process using a combination of moderate steaming temperature and rotation speed yields the highest responses in all dependent variables (DPPH, FRAP, and SDF). When applying a steaming temperature of 150°C and a drum rotation speed of 2 rpm, the predicted maximum DPPH scavenging activities for PAP, PS-PAP, and LS-PAP sheets were 74.32, 69.03, and 69.97%, respectively and the experimental values were 70.69 ± 0.41%, 65.52 ± 0.79%, and 67.63 ± 0.04%, respectively. Additionally, for FRAP, the predicted maximum values for PAP, PS-PAP, and LS-PAP sheets were 272.92, 225.72, and 254.06 µmol ascorbic acid/100 g, while the experimental values were

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268.94 ± 0.33, 219.56 ± 0.73, and 246.41 ± 0.06 μmol ascorbic acid/100 g, respectively. For SDF of PAP, PS-PAP, and LS-PAP sheets, the predicted values were 1.56, 6.26, and 6.58 g/100 g, and the experimental values were 1.63 ± 0.38, 5.89 ± 0.66, and 6.32 ± 0.03 g/100 g.

The experimental values for DPPH (Y1), FRAP (Y2), IDF (Y3), and SDF (Y4) were subjected to multiple regression analysis using response surface analysis to fit a quadratic polynomial equation. The experimental values closely matched the predicted values, indicating an adequate model. The R2 values, adjusted R^2 values, probability values, and lack-of-fit values of the dependent variables are presented in Table 3. The lack-of-fit test showed that the response model accurately predicted the dependent variables. Graphical optimization was performed to determine the optimal conditions of the drum drying process. The desirability value represents the desirable value that maximizes DPPH, FRAP, and SDF. Figure 2 displays the overlaying contour plots that indicate the optimal steaming temperature and rotation speed. Numerical optimization revealed the optimum conditions for the PAP, PS-PAP, and LS-PAP sheets to be 151.88°C at 2.40 rpm, 154.41°C at 2.36 rpm, and 154.16°C at 2.28 rpm, respectively. The desirability plot area represents the range of independent variables within which the desirable values of the dependent variables are achieved. This area accounts for variations or fluctuations in steaming temperature and rotation time. Table 4 presents the predicted response values for DPPH, FRAP, and SDF. The predicted DPPH values for PAP, PS-PAP, and LS-PAP sheets were 74.04, 68.93, and 69.97%, respectively. The experimental DPPH values were 70.69 ± 0.41%, 65.52 ± 0.79%, and 67.63 ± 0.04%, indicating differences of 4.53, 4.95, and 3.34%, respectively. Similarly, for FRAP, the predicted values for PAP, PS-PAP, and LS-PAP sheets were 273.43, 225.93, and 254.04 μmol ascorbic acid/100 g. Comparing these values to the experimental values of 268.94 ± 0.33, 219.56 ± 0.73, and 246.41

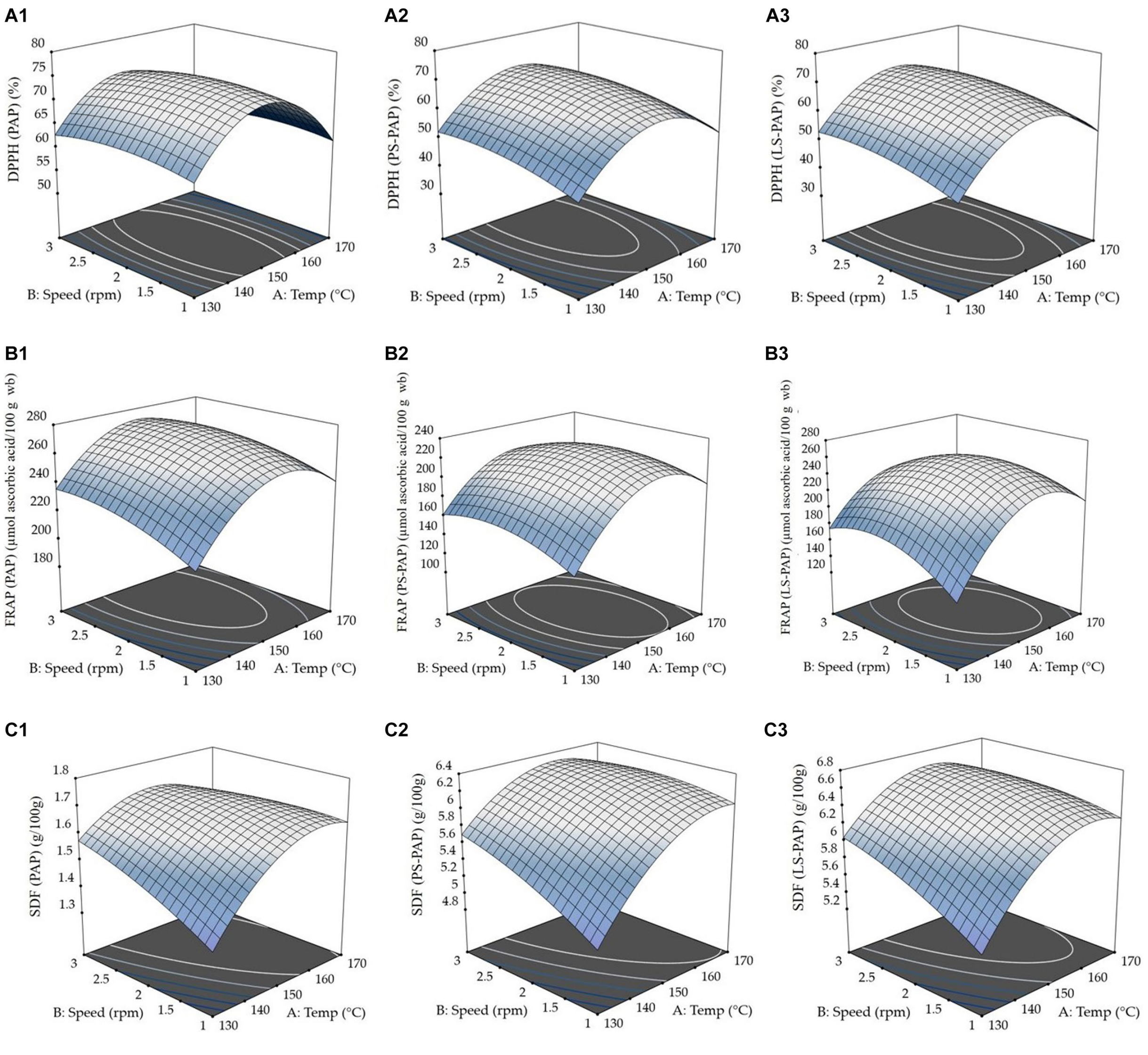

FIGURE 1
Response surface plot of steaming temperature and rotation speed on; DPPH (% scavenging activity) of PAP sheet (A1), PS-PAP sheet (A2), and LS-PAP sheet (A3); FRAP (μmol ascorbic acid/ 100 g) of PAP sheet (B1), PS-PAP sheet (B2), and LS-PAP sheet (B3); and SDF (g/100 g) of PAP sheet (C1), PS-PAP sheet (C2), and LS-PAP sheet (C3).

± 0.06 μmol ascorbic acid/100 g, the differences were 1.64, 2.82, and 3.00%, respectively. Regarding SDF, the predicted values for PAP, PS-PAP, and LS-PAP sheets were 1.70, 6.26, and 6.58, while the experimental values were 1.63 ± 0.38, 5.89 ± 0.66, and 6.32 ± 0.03, indicating differences of 4.06, 5.91, and 3.94%, respectively.

Comparison of optimized drum drying factors for verifying predicted and experimental values of responses

Table 4 presents a comparison of the optimized drum drying factors used for verifying the predicted and experimental values for the antioxidant properties and fiber contents of the different samples. The optimized factors include steam temperature and rotation speed of the drum. For the PAP sheets, the optimal factors were determined to be a steam temperature of 151.13°C and a rotation speed of 2.29 rpm. The experimental values for the antioxidant properties and fiber content were compared to the predicted values. The differences between the experimental and predicted values ranged from 1.46 to 4.88%. These differences indicate a reasonably good agreement between the predicted and experimental values, suggesting that the optimization process was effective in achieving the desired properties. Similarly, for the PS-PAP sheets, the optimal factors were a steam temperature of 153.84°C and a rotation speed of 2.39 rpm. The differences between the experimental and predicted values ranged from 2.73 to 5.08%. While there is some variability between the predicted and experimental values, the overall agreement suggests that the optimized factors had a positive impact on the antioxidant properties and fiber content of the sample. For the LS-PAP sheets, the optimal factors were a steam temperature of 154.15°C and a rotation speed of 2.28 rpm. The differences between the experimental and predicted values ranged from 3.01 to 3.95%. These results indicate a relatively good agreement between the predicted and experimental values, demonstrating the effectiveness of the optimized drum drying factors and suggests that the optimization process was successful in achieving the desired antioxidant properties and fiber content for all three samples. The close agreement between the predicted and experimental values indicates the potential of drum drying as a viable method for producing antioxidant-rich and fiber-rich food products.

The desirability of the optimized formulations using different variables was analyzed based on the response surface methodology (RSM) and the predicted values obtained from the regression models. The desirability function is a tool that combines multiple response variables and provides a single value that represents the overall desirability of a set of conditions. In this study, the response variables include DPPH scavenging activity, FRAP, and SDF content. The goal was to find the optimal combination of steaming temperature and drum rotation speed that maximizes these response variables simultaneously. The desirability function ranges from 0 to 1, where 0 indicates the least desirable conditions, and 1 indicates the most desirable conditions. A desirability value of 1 means that all response variables are at their highest values, representing the best combination of variables. To determine the optimal conditions for the different samples (PAP, PS-PAP, and LS-PAP sheets), the desirability function was used to find the combination of steaming temperature and drum rotation speed that yields the highest desirability value (Table 1). The optimal conditions for the PAP sheets were a steaming temperature of 151.13°C and a drum rotation speed of 2.29 rpm. At these conditions, the predicted values for DPPH scavenging activity, FRAP, and SDF content were 74.04%, 273.43 μmol ascorbic acid/100 g, and 1.70 g/100 g, respectively. The desirability value for these conditions would be calculated based on how close these predicted values are to the ideal values of 100% for DPPH scavenging activity, the highest possible value for FRAP, and the highest possible value for SDF content. A higher desirability value indicates a more optimal combination of variables. The same process was employed for the PS-PAP and LS-PAP sheets, with different optimal conditions and desirability values for each sample. Overall, the desirability function was used to identify the best combination of steaming temperature and drum rotation speed that maximizes the antioxidant and dietary fiber content of the pineapple pomace fortified in crispy mushroom sheets. The reported results suggest that the optimized drying conditions led to high antioxidant properties and dietary fiber content in the dried samples.

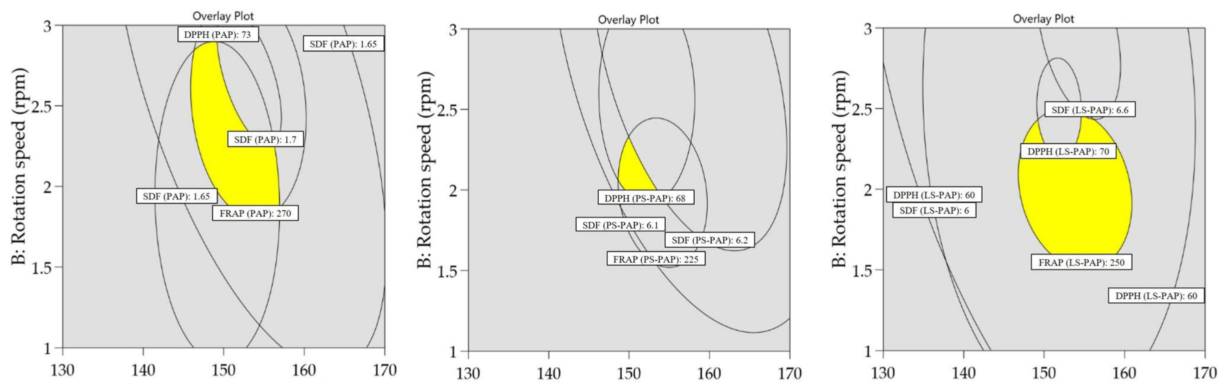

FIGURE 2
Superimpose of the desirable area with the effect of steaming temperature and rotation speed of PAP, PS-PAP and LS-PAP sheets.

TABLE 4 Comparison of optimized drum drying factors for verifying predicted and experimental values of antioxidant properties and fiber content.

Samples	Optimal factors		Responses	Experimental value	Predicted value	Difference (%)
	Steam temperature (°C)	Rotation speed of drum (rpm)				
PAP Sheet	151.13	2.29	DPPH	70.69 ± 0.41	74.32	4.88
			FRAP	268.94 ± 0.33	272.92	1.46
			SDF	1.63 ± 0.38	1.56	4.29
PS-PAP sheet	153.84	2.39	DPPH	65.52 ± 0.79	69.03	5.08
			FRAP	219.56 ± 0.73	225.72	2.73
			SDF	5.89 ± 0.66	6.26	5.91
LS-PAP sheet	154.15	2.28	DPPH	67.63 ± 0.04	69.97	3.34
			FRAP	246.41 ± 0.06	254.06	3.01
			SDF	6.32 ± 0.03	6.58	3.95

PAP sheet, Pineapple pomace sheet; PS-PAP, Oyster mushroom (Pleurotus sajor-caju) (PS) fortified with PAP sheet; LS-PAP, white log (Lentinus squarrosulus Mont) (LS) fortified PAP sheet.

TABLE 5 Physicochemical characterization of drum dried pineapple pomace powder.

Physicochemical parameters	Pineapple pomace (PAP)
a_w	0.75 ± 0.08
pH	4.53 ± 0.17
TA (% citric acid)	1.85 ± 0.25
DPPH (% scavenging activity)	70.13 ± 1.12
FRAP (μmol of ascorbic acid/100 g)	161.85 ± 3.18
IDF (g/100 g)	30.44 ± 0.14
SDF (g/100 g)	0.63 ± 0.03

Values are presented as mean ± standard deviation. TA, titratable acidity; DPPH, (2,2'-Diphenyl-1-picryl-hydrazyl); FRAP, (ferric reducing antioxidant power); IDF, (insoluble dietary fiber); SDF, (soluble dietary fiber).

Physicochemical characterization of optimized pineapple pomace

The physicochemical properties of 'Phulae' pineapple pomace powder subjected to drum drying processing are presented in Table 5. The physicochemical characterization of drum dried PAP powder revealed its potential as a valuable byproduct. The PAP powder was moderately acidic pH of 4.53 ± 0.17 and had considerable titratable acidity (1.85 ± 0.25% citric acid), indicating its potential use as an ingredient in acidic food formulations. Moreover, PAP demonstrated significant antioxidant activity, with a DPPH scavenging activity of 70.13 ± 1.12% and a high FRAP of 161.85 ± 3.18 μmol ascorbic acid/100 g, suggesting its potential as a natural antioxidant source. Additionally, PAP contained substantial amounts of insoluble dietary fiber (30.44 ± 0.14 g/100 g) and smaller amounts of soluble dietary fiber (0.63 ± 0.03 g/100 g), indicating its potential as a dietary fiber source. The results of physicochemical properties especially TDF were compared with the reports of Selani et al. (2014) that indicated that pineapple pomace powder contains 99.2% insoluble dietary fiber and 0.8% soluble dietary fiber. pH and titratable acidity values showed variation with the values reported for PAP powder possibly due to differences in species, environmental factors such as microbial load, enzymatic activity, or biochemistry of fruit (Meena et al., 2022).

Proximate composition of optimized crispy mushroom sheets fortified with pineapple pomace after twin drum drying

The proximate composition of the mushroom sheets with and without PAP after drum drying are presented in Table 6. The sheets exhibited varying levels of dry matter prior to drum drying. After drum drying, the dry matter content increased significantly in all samples, with the PS-PAP and LS-PAP showing the highest contents at 95.17 ± 0.21% and 95.29 ± 0.17%, respectively. The fat contents of both the PS and LS sheets were low (~0.13%) before drum drying; the PAP sheets a slightly higher fat content (0.28 ± 0.01%). After drum drying, the fat content increased in all samples, with the PS-PAP sheets having the highest fat content (1.32 ± 0.03%) followed by the LS-PAP sheets (1.37 ± 0.02%). Protein contents were relatively similar among the samples before drum drying; however, the PS and LS sheets had higher protein contents compared to the PAP sheets. After drum drying, the protein content increased significantly in both PS-PAP (12.44 ± 0.05%) and LS-PAP sheets (13.36 ± 0.08%). Ash contents represent the inorganic mineral compositions. The PS and LS sheets had similar ash percentages before drum drying; however, the PAP sheets had a higher ash content (2.15 ± 0.01%). After drum drying, the ash contents increased in both the PS-PAP (5.77 ± 0.01%) and LS-PAP sheets (5.71 ± 0.01%). Total dietary fiber (TDF) provides an indication of the fiber content in the samples. The PS and LS sheets had comparable TDF percentages before drum drying; however, the PAP sheets had the highest TDF content (35.67 ± 0.18%). After drum drying (Table 6), the TDF content decreased in both PS-PAP (25.38 ± 0.05%) and LS-PAP sheets (27.03 ± 0.12%), possibly due to the effects of the drying process. Carbohydrate contents represent the remaining portions of the samples after accounting for the other proximate components. The PS and LS sheets had similar carbohydrate percentages before drum drying but the PAP sheets had the highest carbohydrate contents (20.62 ± 0.22%). After drum drying, the carbohydrate content increased significantly in both PS-PAP (50.26 ±

TABLE 6 Proximate composition of mushroom samples after drum drying processing.

Samples	Dry matter (%)	Fat (%)	Protein (%)	Ash (%)	TDF (%)	Carbohydrate (%)
PS sheet	10.80 ± 0.14	0.14 ± 0.01	6.28 ± 0.01	0.53 ± 0.001	1.83 ± 0.24	2.02 ± 0.02
LS sheet	10.58 ± 0.03	0.13 ± 0.01	6.34 ± 0.02	0.61 ± 0.01	1.92 ± 0.37	1.58 ± 0.01
PAP sheet	60.65 ± 0.25	0.28 ± 0.01	1.93 ± 0.01	2.15 ± 0.01	35.67 ± 0.18	20.62 ± 0.22
PS-PAP sheet	95.17 ± 0.21	1.32 ± 0.03	12.44 ± 0.05	5.77 ± 0.01	25.38 ± 0.05	50.26 ± 0.90
LS-PAP sheet	95.29 ± 0.17	1.37 ± 0.02	13.36 ± 0.08	5.71 ± 0.01	27.03 ± 0.12	47.82 ± 0.81

PAP sheet, Pineapple pomace sheet; PS-PAP, Oyster mushroom (Pleurotus sajor-caju) (PS) fortified with PAP sheet; LS-PAP: white log (Lentinus squarrosulus Mont) (LS) fortified PAP sheet.

TABLE 7 Microbiological analysis of crispy mushroom sheets supplemented with pineapple pomace.

Microbiological tests	Sample products	
	PS-PAP sheet	LS-PAP sheet
Total plate count (CFU/g)	8.0×10	7.0×10^2
Yeast and Mold (CFU/g)	4.7×10	7.5×10^2
Bacillus cereus (CFU/g)	< 10	< 10
Clostridium perfringens (CFU/g)	< 10	< 10
Escherichia coli (MPN/g)	< 3.0	< 3.0
Salmonella spp. (in 25 g)	ND	ND
Staphylococcus aureus (CFU/g)	< 10	< 10

0.90%) and LS-PAP sheets (47.82 ± 0.81%) and were the highest of all of the sheets.

The drum drying processing had a significant impact on the proximate composition of the mushroom samples. The process led to a considerable increase in the dry matter content of all samples, indicating the removal of moisture during drying. This increase in dry matter is desirable for enhancing the shelf life and stability of the mushrooms. The fat content of the samples increased after drum drying and can also be attributed to a concentration effect caused by moisture removal. The higher fat content in the PS-PAP and LS-PAP sheets may be attributed to the addition of PAP, which likely contributed to the increase in fat content. The protein content significantly increased in both PS-PAP and LS-PAP sheets after drum drying. This increase may be attributed to a concentration effect resulting from the removal of water during drying. The addition of PAP also contributed to the higher protein content in these samples. The ash content, representing the inorganic mineral content, increased in both PS-PAP and LS-PAP sheets after drum drying. The higher ash content in the PAP and the incorporation of PAP in the PS and LS samples likely contributed to this increase. The decrease in TDF content after drum drying in PS-PAP and LS-PAP sheets is intriguing. The drying process might have caused some structural changes in the fiber components, leading to a reduction in TDF. Further analysis is required to understand the specific effects of drum drying on the fiber composition of mushrooms. Carbohydrate contents increased significantly in both PS-PAP and LS-PAP sheet samples after drum drying. This increase is likely due to the concentration effect caused by moisture removal during drying and the higher carbohydrate content in the PAP sheet itself. The proximate composition of the pineapple pomace fortification in mushroom sheets showed higher total dietary fiber. This was in agreement with the proximate composition results of functional pasta extruded by blending pineapple pomace and soy protein floor (Devi et al., 2023). The study evaluated the protein, fat, fiber, carbohydrate, and mineral contents of wheat flour biscuits developed with a functional additive of 5% pomace powder, aiming to create biscuits with health-promoting properties (Thivani et al., 2016). Another study showed apple pomace powder contains high levels of fiber (51%) and carbohydrate (31.24%), but low levels of fat (2.16%) and protein (4.88%) and that the pomace derived from apples can be effectively utilized as a fiber-enhancing ingredient in low-fiber foods, such as yogurt (Meena et al., 2022).

Microbiological quality of crispy mushroom sheets added with pineapple pomace

Table 7 presents the microbiological analysis results of the crispy sheet mushroom sheets supplemented with pineapple pomace. The total plate count provides an estimation of the total viable microbial population present in the samples. The PS-PAP sheets had a TPC of 8.0×10 CFU/g, while the LS-PAP sheets had a slightly higher TPC of 7.0×10^2 CFU/g. These counts indicate a relatively low microbial load in both samples. Yeast and mold counts were also determined in the samples. The PS-PAP sheet had a yeast and mold count of 4.7×10 CFU/g, while the LS-PAP sheet had a higher count of 7.5×10^2 CFU/g. These counts indicate the presence of yeasts and molds in both samples, although the LS-PAP sheet had a slightly higher count. The presence of specific pathogenic bacteria, such as *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, and *Staphylococcus aureus*, in the sheets was also determined. The counts for these pathogens were below the detectable limit (< 10 CFU/g) in both the PS-PAP and LS-PAP sheets, indicating the absence or presence at very low levels of these organisms. *E. coli* is a commonly-tested indicator of fecal contamination, and the Most Probable Number method was used to estimate the *E. coli* levels in the samples. Both the PS-PAP and LS-PAP sheets had *E. coli* levels below 3.0 MPN/g, indicating the absence or very low levels of this pathogen. The presence of *Salmonella* spp., a pathogenic bacterium associated with foodborne illness, was also investigated, and the PS-PAP and LS-PAP sheets tested negative for this pathogen. *Staphylococcus aureus* is another pathogenic bacterium commonly found in food, and counts for this pathogen were below the detectable limit (< 10 CFU/g) in both sheets. A study by Selani et al. (2014) was conducted on the development of an extruded snack product made from pineapple pomace and the authors found that fiber enhancement reduced microbial loads. The microbial counts determined in our study suggest that mushroom sheets supplemented with PAP have low microbiological hazards risk. This low risk was attributed to the minimal moisture content, which likely prevents microbial proliferation (Alp and Bulantekin, 2021). A study by Babaoğlu et al. (2022) found that the inclusion of water extracts from black chokeberry, blackberry, blueberry, and red currant pomace resulted in improved quality characteristics of beef patties. Specifically, the extracts inhibited the growth of mesophilic and psychotropic aerobic bacteria, lactic acid bacteria, and coliforms, thereby enhancing the overall microbial quality of the patties.

Conclusion

The pomace produced from the processing of 'Phulae' pineapples has great potential for the production of new functional snacks due to its physicochemical properties. The use of the Box–Behnken design allowed the determination of optimum parameters for the processing of PAP, PS-PAP, and LS-PAP sheets to achieve maximum DPPH, FRAP, and SDF contents. For the PAP sheet, the optimum parameters were a steaming temperature of 151.13°C and a rotation speed of 2.29 rpm. Similarly, for the PS-PAP sheets, the optimum parameters were 153.84°C and 2.39 rpm, and for the LS-PAP sheets, the optimum parameters were 154.15°C and 2.28 rpm. Furthermore, increasing the temperature and time showed the potential to transform a partial fraction of IDF into SDF. Moreover, the crispy sheet mushroom products were found to be safe, as there was no proliferation of pathogenic bacteria. In conclusion, these crispy sheet mushroom products can be considered functional food, as they provide an alternative protein snack fortified with dietary fiber and could be scaled up for production.

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

PS: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. MN: Supervision, Writing – review & editing. SS: Conceptualization, Resources, Supervision, Writing – review & editing.

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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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Mobile robotics in smart farming: current trends and applications

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In recent years, precision agriculture and smart farming have been deployed by leaps and bounds as arable land has become increasingly scarce. According to the Food and Agriculture Organization (FAO), by the year 2050, farming in the world should grow by about one-third above current levels. Therefore, farmers have intensively used fertilizers to promote crop growth and yields, which has adversely affected the nutritional improvement of foodstuffs. To address challenges related to productivity, environmental impact, food safety, crop losses, and sustainability, mobile robots in agriculture have proliferated, integrating mainly path planning and crop information gathering processes. Current agricultural robotic systems are large in size and cost because they use a computer as a server and mobile robots as clients. This article reviews the use of mobile robotics in farming to reduce costs, reduce environmental impact, and optimize harvests. The current status of mobile robotics, the technologies employed, the algorithms applied, and the relevant results obtained in smart farming are established. Finally, challenges to be faced in new smart farming techniques are also presented: environmental conditions, implementation costs, technical requirements, process automation, connectivity, and processing potential. As part of the contributions of this article, it was possible to conclude that the leading technologies for the implementation of smart farming are as follows: the Internet of Things (IoT), mobile robotics, artificial intelligence, artificial vision, multi-objective control, and big data. One technological solution that could be implemented is developing a fully autonomous, low-cost agricultural mobile robotic system that does not depend on a server.

KEYWORDS

mobile robotics in agriculture, smart farming, path planning in agriculture, IoT in agriculture, unmanned ground vehicle in agriculture, precision agriculture, intelligent agriculture

1. Introduction

In recent years, the global population has increased unprecedentedly, leading to significant changes in food demand (Dhumale and Bhaskar, 2021). As we move into the future, it is expected that the demand for food will continue to rise, driven by factors such as population growth, urbanization, and changing dietary preferences. In addition, the effects of climate change have also impacted food demand and supply, creating new challenges for the food industry (Dutta et al., 2021). In Springmann et al. (2018), it is mentioned that by 2050, the food chain might increase production by 50%. Besides, the FAO shows that the world population will reach approximately 10 billion by that year (Ahmed et al., 2018). This population increase affects the environmental conditions, which changes the harvesting process forcing farmers to use fertilizers and pesticides (Shafi et al., 2019). The residuals of those chemical pollutants contaminate water (Rajeshwari et al., 2021). Another concern is the nutritional outcome that offers food since the previous statement that the environmental condition worsens, creating floods and droughts. Therefore, humans are not receiving enough nutrients to be healthy by eating processed food, requiring pills and supplements (Mostari et al., 2021). The Intergovernmental Panel on Climate Change (IPCC) warns that global warming reduces the nutritional value of crops due to the intensive use of fertilizers to boost crop yields; they also predict that in the incoming years, people might suffer from zinc deficiency, causing even their psychological and cognitive disorders (Ryan et al., 2021).

Technology in the food production industry is a significant challenge that impedes progress and innovation in this critical sector. With the rapidly growing global population and increasing demand for food, it has become imperative to adopt technological advancements to improve food production and distribution (Ferrag et al., 2021). However, in many parts of the world, particularly in developing countries, technology in food production still needs to be improved, resulting in low productivity, high food losses, and reduced efficiency. Given that a big part of food production is from developing countries, exists a lack of advanced agricultural technologies (Khan et al., 2021). They face significant financial constraints and limited access to modern technologies, which can impede their ability to improve their food production processes. This concern also extends to the education and training of the workforce, who may not have the knowledge and skills to operate and maintain technological tools and equipment effectively (Xuan, 2021).

To mitigate the concerns mentioned above about food supply, FAO proposes four bullet points to guarantee food quality in the incoming years, which they closely related to the use of technology since information plays a fundamental role in ensuring the economic and sustainability impacts of new cutting-edge techniques in the food production process (Mooney, 2020).

Implementing emerging technologies in agriculture is often called smart farming, which aims to improve productivity, efficiency, and sustainability (Raj et al., 2021). In Belhadi et al. (2021), mention that smart farming might use trend technologies such as robotics, artificial intelligence, and the IoT. Therefore, these devices can gather data from crops to extract intrinsic knowledge from plants to improve agricultural decision-making and reduce environmental impact (Megeto et al., 2021). However, the full exploitation of the potential of smart farming presents several challenges and technical, socio-economic, and administrative constraints (Mengoli et al., 2021). Works such as Ahmed et al. (2016), Jawad et al. (2017), Bermeo-Almeida et al. (2018), Kamilaris and Prenafeta-Boldu (2018), and Rahmadian and Widyartono (2020) present broad approaches to smart farming and trend technologies without focusing only on robots. These studies do not include a detailed discussion of the tools and techniques used to develop the different mobile systems and their level of maturity. It is relevant to discuss the use of mobile robotics in smart farming from different perspectives and describe their corresponding nuances.

This article stands out from others of a similar nature because it offers a broad overview of the challenges and opportunities presented by precision agriculture and robotic farming. The article focuses on the use of robotics and precision agriculture in agriculture 4.0 and provides a detailed description of the many types of agricultural robots used, as well as the techniques and hardware used for their operation and monitoring. Additionally, the article highlights the areas where literature is least developed and suggests potential solutions to address these challenges. Future trends in precision agriculture and robotics are also discussed, including the use of multi-objective control algorithms and artificial intelligence in low-cost mobile robots for planning the best path while accounting for energy efficiency, soil type, and obstacles, as well as for evaluating and managing pests and diseases that affect crops.

This work aims to present an overview of mobile robotics implemented for agricultural production related to smart farming techniques. The main contribution of this work is showing the existing frameworks, tools, and applications where robots are currently used. Also, it presents shortcomings in smart farming applications, which might provide future trends in robots. The rest of the manuscript is structured as follows: Section 2 gives the smart farming background and provides a detailed overview of the leading mobile robots with existing technologies. Section 3 presents the discussion highlighting the technical and socio-economic obstacles to successfully integrating mobile robotics in agriculture. Section 4 presents the future trends related to mobile robotics in agriculture. Finally, Section 5 presents the conclusions.

2. Research methodology

A systematic literature review (SLR) was performed to manage the diverse knowledge and identify research related to the raised topic (Ahmed et al., 2016), especially to investigate the status of mobile robotics in precision agriculture. In particular, we searched for papers on "mobile robotics" with the term "agriculture 4.0" in the title, abstract or keywords. Prior to the SLR, a review protocol was defined to ensure a transparent, high quality and comprehensive research process (Page et al., 2021) including three steps: formulating the research questions, defining the search strategy, and specifying the inclusion and exclusion criteria. The preferred reporting approach for systematic reviews and meta-analyses (PRISMA) was used to conduct the SLR.

2.1. Review protocol

Before starting the bibliographic analysis, a review protocol was defined to identify, evaluate and interpret the relevant results of the research topic (see Table 1). The first step was to formulate research questions to identify the studies published on the subject of interest from different approaches. The appropriate keywords were then identified order to formulate search strings to obtain relevant information using four databases: IEEE Xplore, Web of Science, Scopus, and ScienceDirect. To refine the search results, inclusion and exclusion criteria were defined to evaluate the content of the publications and used as a preliminary filter of the metadata sources and limit the scope of the research.

TABLE 1 Review protocol for SLR.

Review questions	RQ1: How are mobile robotics used in agriculture?
	RQ2: What technologies, methods, and tools are being used in agricultural fields?
	RQ3: What are the main challenges of multi-objective control in agriculture 4.0?
Selection criteria	**Inclusion criteria:**
	- Journal and conference articles.
	- Research published during the period from 2016 to 2022.
	- Studies that provide answers to the research questions posed.
	- Literature focused on the application of mobile robotics in agricultural field activities.
	- Early access articles.
	Exclusion criteria:
	- Summaries of events and seminars.
	- Literature focused on the application of mobile robotics to post-field stages.
	- Publications not available in full text.
	- Articles in languages other than English.
Literature search	**Sources:** IEEE Xplore, Web of Science, Scopus, and ScienceDirect for academic literature.
	Search strings were used: "agriculture" AND "UGV" AND "Path Planning" OR "Multi-objective Control" OR "Path Tracking" OR "Precision Agriculture" OR "Smart Farming" OR "Agriculture 4.0" OR "IoT"

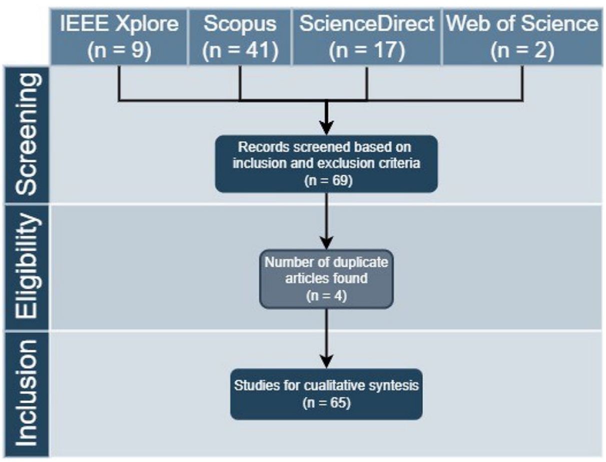

FIGURE 1
Three-steps evaluation of literature search process (PRISMA).

After performing the SLR, 69 research articles were obtained on the proposed topic. After the PRISMA selection and eligibility steps with the help of the Mendeley bibliographic reference manager, similar files were identified and eliminated, leaving a total 65 research papers, as can be seen in Figure 1.

2.2. Trends in agriculture

The distribution of the 65 articles by year, about 38% of the most recent scientific papers were published in 2021, reflecting the considerable progress of agriculture in the context of mobile robotics, although the pace can still be considered slow compared to other domains such as healthcare, the manufacturing, the mining, the automation, the energy, among others (Araújo et al., 2021).

Figure 2 gives the breakdown of publications on the five most common activities carried out by agriculture 4.0 and the type of mobile robot employed. The multiple tasks in the field category include activities such as row recognition and tracking, obstacle detection and avoidance, and information gathering and reporting in both outdoor and greenhouse agriculture.

According to the International Federation of Robotics (IFR), the top five service robot applications for professional use sold during 2019 and 2020 are: transportation and logistics, professional cleaning, medical robotics, hospitality, and agriculture (International Federation of Robotics, 2021). Figure 3 gives the percentage of robots employed in each of these areas.

3. Background and related works

Smart farming is a technique that uses advanced technology to optimize yield and efficiency in agricultural production. In Lohchab et al. (2018), explored the application of IoT technologies in smart agriculture. Subsequently, in a 2020 review article, Sharma et al. (2020) focused on the use of artificial intelligence and machine learning in smart agriculture. Furthermore, in a 2021 review article, Ratnaparkhi et al. (2020) discussed the implementation of sensor technologies and Geographic Information Systems (GIS) for smart agriculture. Finally, in a recent 2022 review article, Botta et al. (2022) examined the integration of robotics and automation in smart agriculture. Some topics that very little has been addressed in smart agriculture are: the integration of smart agriculture with the circular economy and environmental sustainability, the development and application of artificial intelligence and machine learning technologies in pest and disease identification and management, increased focus on optimizing water use and irrigation management in response to climate change and limited water availability, improved connectivity and interoperability of systems to facilitate large-scale adoption and implementation, and the development of specific low-cost solutions for small farms and rural communities in developing countries to improve food security and reduce rural poverty.

3.1. Smart farming

Smart farming is based on the information provided by sensors placed on an agricultural field (Ahmed et al., 2016); Machine Learning (ML) models could learn patterns to support the farmers' decision-making (Mammarella et al., 2020; Shorewala et al., 2021). These sensors joined with a microcontroller sending data constantly, are considered part of the IoT. Besides, data might be processed in big servers allocated in the cloud (cloud computing). However, IoT devices are often a rigid solution since

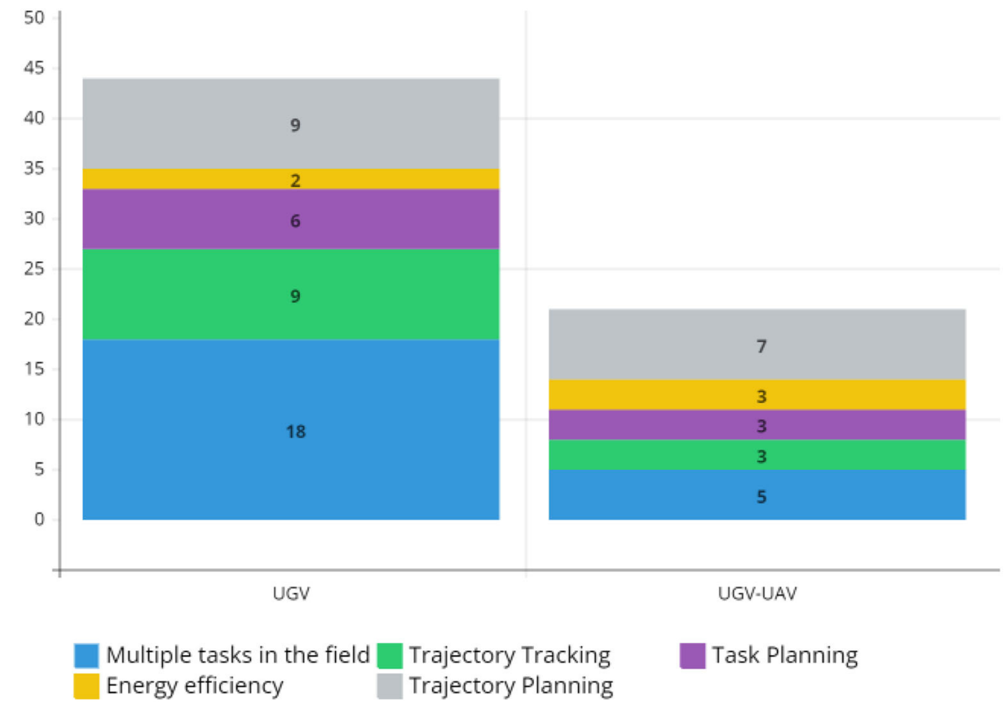

FIGURE 2
Mobile robotics activities in agriculture.

they are placed in a single location. Therefore, Autonomous Robotic Systems (ARS) can walk around crops taking data from the whole farm and providing accurate information (Ozdogan et al., 2017; Kamilaris and Prenafeta-Boldu, 2018). This combination of sensors, data analysis, and robots provides farmers with a smart farming application with diverse tools to address challenges related to productivity, environmental impact, food safety, crop losses, and sustainability. The objectives of smart farming are to increase crop yields, minimize costs, and improve product quality through using a modern system (Araújo et al., 2021). In the last years, with technological evolution, different types of sensors have been developed that make it possible to collect data in almost any location, allowing real-time monitoring of agricultural fields without wiring. Therefore, the three leading technologies that contribute significantly to this field are as follows:

- **Drones:** These are small flying robots commonly used for crop monitoring, food infrastructure inspection, supply chain monitoring, and food safety surveillance (Costa et al., 2021).
- **Autonomous tractors:** These are generally Unmanned Ground Vehicles (UGV) incorporating sensors and actuators that enable crop monitoring, irrigation, harvesting, and disease control (Lisbinski et al., 2020).
- **Software for decision making:** These are platforms where data acquired by drones and/or UGV sensors are visualized and analyzed. They generally provide information on weather, soil, crop yields, and other factors relevant to agricultural production to improve decision-making (Ojeda-Beltran, 2022).

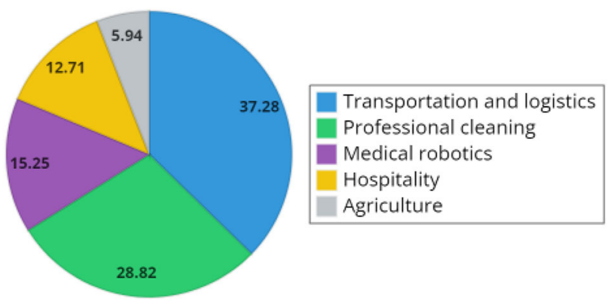

FIGURE 3
Percentage of top five applications of service robots in 2020.

3.2. Mobile robotics in agriculture

The emerging field of agricultural mobile robotics is UGV and UAV (Prakash et al., 2020). The main applications of mobile robotics in farming are:

- Identify the state of the crop and corresponding application of chemical products, fumigation, or harvesting, as required by the fruit or plant.
- Mobile handling through collaborative arms (harvesting, fruit handling).
- Collection and conversion of helpful information for the farmer.
- Selective application of pesticides and avoidance of food waste.

UGV and UAV have limited available power. Therefore, their design and control optimization is paramount for their application in smart farming. Therefore, research on the cooperation between UGV and UAV is being carried out to cover large agricultural areas. These autonomous robots are intelligent machines capable of performing tasks, making decisions, and acting in real-time with a high degree of autonomy (Rahmadian and Widyartono, 2020). Interest in mobile robotics in agriculture has grown considerably in the last few years due to its ability to automate tasks such as planting, irrigation, fertilization, spraying, environmental monitoring, disease detection, harvesting, and weed and pest control (Araújo et al., 2021). Furthermore, mobile robotics in smart farming uses a combination of emerging technologies to improve the productivity and quality of agricultural products (Bechar and Vigneault, 2016).

UGV are robots that control can be remote (controlled by a human operator through an interface) or fully autonomous (operated without the need for a human controller based on AI technologies) (Araújo et al., 2021). The main components of UGV are locomotive, manipulator and supervisory control systems, sensors for navigation, and communication links for information exchange between devices. The main locomotion systems used are wheels, tracks, or legs. To properly operate UGV in the field, they must meet size, maneuverability, efficiency, human-friendly interface, and safety requirements. Table 2 summarizes the diverse range of UGVs designed for agricultural operations.

The main issue of mobile robotics in agricultural fields is to perform multiple tasks (obstacle avoidance, tracking, path planning, crop data collection, disease detection, among others) autonomously with reduced hardware for low-cost robots that can be acquired and implemented by farmers. Most UGV presented above have a wheeled locomotion system, offering easy construction and control. Some UGV incorporate low-cost computer vision systems, i.e., using conventional cameras. UGV might employ heuristic algorithms still in the conceptual or prototyping phase. Due to the limitations of UGV and to cover larger areas and less time, in the last years, the UGV-UAV collaboration has been developed (Khanna et al., 2015). The UGV operates in the areas selected by the UAV, which also cooperates in the generation of 3D maps of the environment with centimeter accuracy; however, merging the maps generated by UAV and UGV in an agricultural climate is a complex task since the generated maps present inaccuracies and scale errors due to local inconsistencies, missing data, occlusions, and global deformations (Gawel et al., 2017; Potena et al., 2019). Table 3 reviews some collaborations between UGV and UAV in smart farming.

Most collaborative systems between UAV and UGV are in the conceptual (simulation) phase.

3.3. Multi-objective control in smart farming

Agricultural systems use and produce energy in the form of bioenergy and play a vital role in the global economy and food security. Modern agricultural systems might therefore consider economic, energy, and environmental factors simultaneously (Banasik et al., 2017). Multi-objective control is an important tool in smart farming to simultaneously run and optimize multiple objectives, such as productivity, water use efficiency, product quality, and economic profitability. Some cases of multi-objective control in smart farming are presented in Table 4, which shows their primary function, control techniques, and the hardware deployed. However, there are few studies since this topic is new in smart farming applications with mobile robots. Furthermore, path planning is an essential application of smart agriculture that focuses on optimizing routes and movements of agricultural machinery to improve efficiency and reduce production costs (Nazarahari et al., 2019).

Another application of multi-objective control in path planning is the optimization of fertilization and pesticide application in crops. According to a study by Zhao et al. (2023), multi-objective control can optimize the routing of pesticide and fertilizer application machinery to reduce the number of inputs used and improve application efficiency. In addition, multi-target control can also improve product quality and reduce environmental pollution by accurately applying crop inputs.

Finally, a study proposes a Residual-like Soft Actor-Critic (R-SAC) algorithm for agricultural scenarios to realize safe obstacle avoidance and intelligent path planning of robots. The study also proposes an offline expert experience pre-training method to improve the training efficiency of reinforcement learning. Experiments verify that this method has stable performance in static and dynamic obstacle environments and is superior to other reinforcement learning algorithms (Yang et al., 2022).

4. Discussion

With the information mentioned above about mobile robots in smart farming, this section aims to show the future steps in this research field related to its challenges. Given the new UGV and UAV trends in Table 2, the multi-objective control has yet to be widely explored in smart farming applications. It might be due to its complex setup and the expensive computational resources needed. However, multi-objective applications might be doable in incoming robots with the increasing microcontrollers and microprocessor development. Conversely, IoT devices that collect data from farms are extensively deployed in several applications. However, there are new concerns about their confidentiality and the risk that data is exposed when traveling by communication channels (Pylianidis et al., 2021).

Smart farming needs final devices with robust systems working in harsh conditions in outdoor scenarios. However, several works have shown prototypes with their tentative functionalities. Building robots may need several debugging rounds to solve issues with the hardware and software. Consequently, since the robot links people and plants, farmers, considered experts in smart farming, must work closely with the robot's developer. However, the variety of plant and crop species makes it challenging to develop a multi-task robot (Selmani et al., 2019).

The main challenges and future research for deploying smart farming are presented. The present study sought to articulate mobile robotics with smart farming. Looking at Table 4, it can be seen that multi-objective control has not been significantly

TABLE 2 Different types of UGV in agriculture 4.0.

Application	Control	Hardware	Citation
Fusion of color and depth images	Remote-controlled	Kinect v2 sensor and laptop computer	Gai et al., 2020
Artificial vision and weed algorithms	Remote-controlled and autonomous	AgBotII Robotic platform: RGB camera, GPS, PLC, laptop, others	Bawden et al., 2017
Grid-based using Neural Network	Autonomous	Laptop and obstacle sensor	Arindam et al., 2018
Sampling-Based Method and PSO	Autonomous	Laptop computer	Prakash et al., 2020
Non-holonomic A^*	Autonomous	An all-terrain vehicle developed by Polaris with LiDAR, IMU, and GPS	Zhang et al., 2019
D–S evidence theory	Autonomous	Laptop, camera, and laser scanner	Zhao et al., 2019
Genetic algorithm	Autonomous	Laptop	Tsiogkas and Lane, 2018
Automated Deployment of IoT Networks	Autonomous	Pioneer 3-AT, Raspberry Pi B2, IMU, GPS, Arduino Nano, and laptop	Romeo et al., 2020
UTR control using VSC and Hyper Schemes, and Fuzzy potential motion	Autonomous	Computer	Banihani et al., 2021
Heuristic path planning	Autonomous	Computer	Wang et al., 2021
Grid-based algorithms Dijkstra, A^*, and the sampling-based algorithms RRT and RRT^*	Autonomous	Jackal from Clearpath Robotics with Jetson TX2, and LiDAR	Pak et al., 2022
Kalman filter estimation	Autonomous	Car-like-type, GPS, a 3D orientation sensor, and KNRm controller	Sun and Liang, 2022
Allan Variance in a Kalman Filter	-	Laptop and GPS	Luo et al., 2019
Ant Colony Optimization with a Probability — based random — walk strategy and an Adaptive waypoints	Autonomous	Laptop	Liu et al., 2022
Robust model predictive control (RMPC)	Autonomous	Husky A200 with IMU, LiDAR, laptop	Khan et al., 2022
Traveling salesman problem (TSP) enhanced with — Coverage path planning (CPP)	Autonomous	Computer	Xie and Chen, 2020
Generation optimal polynomial trajectories	Autonomous	Rover Dedalo	Gentilini et al., 2021
Compound Fuzzy Control	Autonomous	Jingguan PZ-60 with STM32 controller, laptop, and position sensors	Li et al., 2020
A^* Algorithm	Manual controlled	The Kinect sensor, and laptop	Nerlekar et al., 2022
Dynamic analysis of skid-steering tracked vehicles	Autonomous	Robot smaller than a standard tractor	Tazzari et al., 2020
Auto-guidance algorithm	Manual controlled	Stereo camera, angle sensor, electric power steering, GPS, and workstation ZBOX QK7P3000	Changho et al., 2021
Voronoi diagram	Autonomous	Multi-robot	Kim and Son, 2020
Algorithm row — change maneuver	Autonomous	Robotic platform with LiDAR	Mengoli et al., 2021
Machine vision algorithms	Wheels	RGB camera, and laser distance sensor	Berenstein and Edan, 2018
Harvesting	Convolutional neural networks	Platform Vegebot with camera, UR10 controller, and laptop	Birrell et al., 2020
Smart irrigation	Teleoperation	NodeMCU, water level sensor, Arduino, temperature and humidity sensor	Srinivas and Sangeetha, 2021

explored in smart farming. One of the reasons could be that applying advanced technologies with complex operations can be costly. Hence, the development of these technologies in smart farming should increase in the coming years. Also, the IoT is widely deployed in agriculture for crop monitoring and tracking. Therefore, it can be said that IoT is a research trend within smart farming. However, only a few studies have considered data security and reliability, scalability, and interoperability when developing a smart farming system (Pylianidis et al., 2021).

The results presented also show that most of the use cases are in the prototype phase. One possible reason could be that smart farming links people, animals, and plants making it more difficult than creating systems for non-living things. Another reason could be that the technology is due to the transdisciplinarity of this field, and therefore for the development of intelligent systems, farmers should be familiar with these technologies. Finally, the variety of plant and crop species makes implementing technology in agricultural fields complex (Selmani et al., 2019). The results

TABLE 3 Collaborations between UGV and UAV in smart farming.

Primary function	Techniques used	Hardware	Citation
Monitoring and drone landing	Re-configurable chassis	Solar panels	Quaglia et al., 2018
Optimal control for the refueling of UAV	Non-linear optimal control	Computer	Rucco et al., 2017
UAV/UGV trajectory planning	Partial Differential Equation (PDE)	Computer	Radmanesh et al., 2021
	Energy-constrained minimization	Computer	Edmonds et al., 2021
Path-following of UGV/UAV	Null Space control	Computer	Bacheti et al., 2021
Ground station for UAV/UGV	Improved Ant Colony algorithm	Commercial UAV/UGV, communication module, and Computer	Liang et al., 2021

TABLE 4 Multi-objective control in agriculture 4.0.

Primary function	Techniques used	Hardware	Citation
Energy management	Multi-objective genetic algorithm (MOGA)	Computer	Shamshirband et al., 2015
Crop pattern optimization	Crow Search Algorithm (CSA) and Particle Swarm Optimization (PSO)	Computer	Jain et al., 2021
Optimization of water-food-energy	Fuzzy multi-objective programming model	Computer	Li et al., 2019
Path planner	Non-dominated Sorting Genetic Algorithm using Reference Point Based (NSGA-III)	Computer	Azimi-Mahmud et al., 2019
	MP-PSOGA algorithm	Computer, and UAV	Zhai et al., 2018
	Combinatorial multi-objective	MGV and UGV	Chirala et al., 2021
	Multi-objective particle swarm optimization (CMOPSO)	Computer	Mac et al., 2017
Irrigation	General Algebraic Modeling System (GAMS)	Computer	Galán-Martín et al., 2017

also show that most systems developed are for free-range farms. In addition, it is also evident that research is limited to soil management, fruit detection, and crop quality management. With this, it is corroborated that work must be done on research and development of systems that guarantee the deployment of smart farming at affordable costs. The natural complexity of agricultural fields presents a number of obstacles that prevent the full integration of mobile robotics in smart farming. Therefore, from the analysis, blockages at the technical and socio-economic levels have been identified and classified.

4.1. Technical roadblocks

- **Interoperability.** To establish effective communication between heterogeneous devices, they need to be interconnected, and interoperable (Aydin and Aydin, 2020).
- **Dataquality.** Lack of decentralized systems impedes the deployment of smart farming (Liu et al., 2022).
- **Hardware.** A suitable casing must be constructed that is robust and durable enough to withstand actual field conditions (Villa-Henriksen et al., 2020).
- **Power sources.** A proper energy-saving scheme is necessary as instant battery replacement is complicated. A possible solution to optimize power consumption is using low-power hardware and proper communications management (Jawad et al., 2017).
- **Wireless architectures.** Wireless communication networks and technologies offer several advantages in terms of low cost, wide area coverage, network flexibility, and high scalability (Brinis and Saidane, 2016).
- **Security.** The nature of agricultural fields leads to risks to data privacy, integrity, and availability (Chen et al., 2017).
- **User interface.** Most graphical user interfaces are designed so that only experts can use them (Del Cerro et al., 2021).

4.2. Socio-economic roadblocks

- **Costs.** Costs associated with adopting robotic technologies and systems are the biggest drawback to deploying smart farming (Sinha and Dhanalakshmi, 2022).
- **Return on investment.** When implementing new technologies, farmers are concerned about the payback time and the difficulties in assessing the benefits (Miranda et al., 2019).
- **Gap between farmers and researchers.** Farmer involvement is paramount to the success of smart farming. Farmers face many problems during the production process that technology could solve (Bacco et al., 2019).

Finally, in Charatsari et al. (2022) discusses the importance of responsibility in the process of technological innovation in the agrifood industry. It highlights the need to consider not only technical aspects but also social implications and societal values when introducing innovative technologies. The authors argue that the perception of responsible innovation is limited in various industrial sectors, making it challenging to implement responsible innovation approaches. The complexity of responsible innovation in the agrifood industry requires addressing the multiple scales and levels of interaction between actors and the constant evolution of agrifood systems. Therefore, the article emphasizes the need to adopt responsible innovation practices that consider the social, ethical, and environmental implications of technological innovations in the industry.

5. Future trends

The upcoming initiatives related to using robots represent significant improvements in smart farming. Government initiatives, public-private sectors, and research work in this field might contribute to establishing the right conditions to add new hardware to crops. However, there are some challenges to consider when developing mobile robots in agriculture such as: navigation on uneven terrain (loose soil and unpredictable obstacles) without damaging plants or compromising their own safety, energy efficiency so that they can operate for long periods of time avoiding constant human intervention, crop manipulation, integration with farm management systems and adaptability to different crops and conditions.

For instance, a robotic system can be developed for smart farming, starting from a basic architecture with few components and simple functionality that allows the gradual addition of features and functionality to create a complex system. Future trends in smart farming involve using multi-objective control algorithms and artificial intelligence in low-cost mobile robots to plan the best trajectory considering energy efficiency, soil type, and obstacles while monitoring crop growth and assessing and controlling crop pests and diseases. To ensure good connectivity and live transmission of crop data, 5G technology needs to be widely explored. 5G technology minimizes internet costs and increases information management by remotely performing accurate inspections of agricultural fields (Abbasi et al., 2021). Finally, blockchain, combined with IoT and other technologies, should be applied to address the challenge of information privacy and security (Bermeo-Almeida et al., 2018).

As seen in the tables in the previous sections, most of the UGV have a computer, which increases the cost of this type of robots. Table 5 shows several state-of-the-art boards that could deploy smart farming at affordable prices for farmers.

Finally, in Figure 4, we can see the future of agriculture, for which a correct 5G network deployment and path planning/tracking is essential. Artificial intelligence, machine learning, machine vision, IoT, and cloud computing are needed in each of the activities carried out in agricultural fields.

6. Conclusions

Growing concerns about global food security have accelerated the need to incorporate mobile robots in agriculture. The scientific community and researchers are integrating disruptive technologies into conventional agricultural systems to increase crop quality and yields, minimize costs, and reduce waste generation. This article analyzes the current state and challenges of smart farming. Considering the impact of farming on climate change and healthy food production, it is vital to provide the agricultural sector with low-cost, functional mobile robots. Research questions were posed and answered regarding the use of mobile robotics in agriculture, the technologies, methods, and tools used in agricultural fields, and the main challenges of multi-target control in this area. Several conclusions were drawn, such as the integration of scalable mobile robots incorporating efficient systems. It should be noted that most cases address a specific problem and are in the prototype phase.

From the SLR conducted, it was identified that research on the following topics is limited:

- The implementation of digital twins for robot-based production lines
- Ingenious software project management while narrowing the impact aspect.
- Blockchain in agriculture.
- Context-aware wireless sensor network suitable for precision agriculture.
- Internet of Things (IoT) for smart precision agriculture and farming in rural areas.
- Semantic and syntactic interoperability for agricultural open-data platforms in the context of IoT using crop-specific trait.
- Multi-objective path planner for an agricultural mobile robot in a virtual and real greenhouse environment.
- Closing loops in agricultural supply chains using multi-objective optimization.
- New control approaches for trajectory tracking and motion planning of unmanned tracked robots.

These areas require further research to improve the efficiency and effectiveness of precision agriculture. Likewise, the information gathered in this article makes it clear that the emerging fields of research are:

- **Autonomous navigation.** Planning, tracking of trajectories, and task planning should be considered in this area.
- **Energy efficiency.** Good navigation autonomy is not the only thing that must be taken into account, but also the design and all components that make up the mobile robot since its size and cost directly influence the deployment of smart farming.
- **Communication.** Due to the number of devices involved in smart farming, middleware that improves communication between field devices and the station is important to ensure the reliability and security of information.

The interdependence of these challenges means that a practical solution must be sought with a suitable compromise between the theoretically optimal path that facilitates information exchange and overall system energy optimization. Moreover, the following questions must be considered: the kinematic and dynamic design of the mobile robot, the terrain traversability, the computational complexity of the various algorithms to ensure real-time performance, the use of sensors and low-energy control boards, and the sending and receiving of information. It also identifies the leading technical and socioeconomic obstacles that must be overcome to deploy smart farming successfully. We can see leaps and bounds being made in this area, but there is still a long way to go to mitigate the impact of farming on the environment in the coming years. Finally, one of the areas to be investigated is multi-objective heuristic optimization for autonomous navigation, communication, and energy efficiency of mobile robots.

TABLE 5 Boards for agriculture 4.0.

Feature	Description
Raspberry Pi 4	Model: B+
	System on a chip: Broadcom BCM2711
	CPU:1.5 GHz quad-core processor with Cortex-A72 arm
	GPU: VideoCore VI
	Memory: 1/2/4GB LPDDR4 RAM
	Connectivity: 802.11ac Wi-Fi / Bluetooth 5.0, Gigabit Ethernet
	Video and sound: 2 x micro-HDMI ports supporting 4K@60Hz displays via HDMI 2.0, MIPI DSI display port, MIPI CSI camera port, 4-pole stereo output and composite video port.
	Ports: 2 x USB 3.0, 2 x USB 2.0
	Power Supply: 5V/3A via USB-C, 5V via GPIO header
	Expansion: 40-pin GPIO header
Jetson Nano	Model: B01
	CPU:ARM A57 quad-core
	GPU: NVIDIA Maxwell 128-core
	RAM: 4 GB of 64-bit LPDDR4
	Video Encode: 4K @ 30 | 4x 1080p @ 30 | 9x 720p @ 30 (H.264/H.265)
	Video Decode: 4K @ 60 | 2x 4K @ 30 | 8x 1080p @ 30 | 18x 720p @ 30 (H.264/H.265)
	Connectivity: Gigabit Ethernet 10/100/1000
	Power Supply: Micro-USB 5V 2A, Barrel connector 5V 4A
	Inputs and Outputs: 4x USB 3.0, USB 2.0 Micro-B, HDMI/DisplayPort, GPIO, I2C, I2S, SPI, UART, Two MIPI-CSI camera connectors, Fan connector, PoE connector.
Arduino Portenta	Model: H7
	Microcontroller:STM32H747XI Dual Cortex®-M7 + M4 32bit low-power Arm® MCU
	Radio module: Murata 1DX dual WiFi 802.11b / g / n 65 Mbps y Bluetooth 5.1 BR / EDR / LE
	Secure element: NXP SE0502
	Power supply board (USB/VIN): 5 V
	Compatible battery: Single cell Li-Po, 3.7 V, 700 mAh minimum
	Circuit operating voltage: 3.3 V
	Consumption: 2.95 μA in standby mode
	Display connector: MIPI DSI and MIPI D-PHY host for interfacing with a large low pin count display
	GPU:Chrom-ART Graphics Hardware Accelerator^{TM}
	Timers: 22x timers and watchdogs
	UART: 4 ports (2 with flow control)
	PHY Ethernet: 10/100 Mbps (through expansion port only)
	SD card: Interface for SD card connector (only through expansion port)
	Operational temperature: −40 °C to +85 °C
	High density connectors: Two 80-pin connectors will expose all on-board peripherals to other devices
	Camera interface: 8 bits, up to 80 MHz
	ADC: 3 × ADC with 16-bit max. resolution (up to 36 channels, up to 3.6 MSPS)
	DAC: 2 × 12 bits (1 MHz)
	USB-C: Host/device, DisplayPort output, high/full speed, power supply

Finally, numerous international political organizations play a crucial role in spreading awareness of the technologies involved in precision agriculture and advocating for their successful implementation. These organizations are:

FIGURE 4
Future of agriculture.

- The FAO promotes the use of advanced agricultural technologies through programs and projects, providing technical assistance, training, and resource access for farmers.
- The European Union (EU) supports agricultural modernization and the adoption of innovative technologies in the industry through its Agricultural Common Policy (ACP). Additionally, the UE funds research and development projects in precision agriculture, agricultural robotics, and digital solutions to increase efficiency and sustainability.
- The Department of Agriculture (USDA) of the United States places emphasis on the adoption of cutting-edge agricultural technologies. The USDA supports the implementation of precise agriculture systems, the integration of sensors and IoT devices into agricultural operations, and the promotion of digitalization in the industry through its funding and grant program.
- The focus of AGRA is to encourage the use of contemporary agricultural technologies across the African continent. AGRA works in close partnership with governments, regional organizations, and the private sector to increase access to and availability of improved seeds, fertilizers, and digital farming technologies that boost agricultural productivity and sustainability.
- The World Economic Forum (WEF) has established initiatives and projects to advance precision agriculture. The WEF brings together many actors-including political leaders, business executives, and members of civil society-through its platform “Shaping the Future of Food Security and Agriculture” to develop innovative and collaborative solutions that foster the digital transformation of agriculture.

These political organizations play a crucial role in the spread of advanced agricultural technologies, and they are actively working to promote the adoption of “agriculture 4.0” on a global scale with the aim of enhancing the efficiency, productivity, and sustainability of the agricultural sector.

Author contributions

JVS supervised this project. PR-M and JS contributed in this project. DY-P made the first version of the article under the guidelines of the other authors; likewise, he made the corrections to the observations made by the reviewers and shared them with the other authors for their respective review and subsequent approval. 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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Key implications on food storage in cold chain by energy management perspectives

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Over the past decades, increase in food shortages and food costs with the drought caused by population growth and climate change, it becomes more important to prevent food losses with the widespread use of cold storage. That's why many countries provide incentives for the widespread use of cold stores. However, with cold storages becoming more widespread, keeping energy consumption under control becomes an important issue. Especially in most developing countries, the status and energy performance of installed cold stores are unknown. In this study, energy consumption data were examined by using data from cold stores from different countries. The relationships between some parameters (capacity, volume, etc.) affecting energy consumption were analyzed for 67 established cold stores. In order to measure the energy consumption data of the system under ideal operating conditions, experiments were carried out in the test room under laboratory conditions and comparisons were made according to the data obtained from the field. The data obtained showed that energy consumption in cold stores increased by up to 30%, depending on operating conditions. Therefore, it is very important that cold stores are inspected by the relevant institutions after they are established and during their operation.

KEYWORDS

food storage, cold chain, energy management, refrigeration, sustainability

1. Introduction

In order to deliver the food to the consumer in the desired quality, the cold chain should not be interrupted in all processes involving cold logistics applications. Deficiencies in the cold chain pose a problem in terms of food safety. Storing the harvested products by maintaining the cold chain will prevent food waste. According to FAO's State of Food and Agriculture Organization (2019) report, around 14 percent of the world's food (valued at $400 billion per year) continues to be lost after it is harvested and before it reaches the shops; while UNEP's Food Waste Index Report (2021) shows that a further 17 percent of our food ends up being wasted in retail and by consumers, particularly in households. According to FAO estimates, the food that is lost and wasted could feed 1.26 billion hungry people every year. Fruit and vegetables (40–50%) are among the wasted foodstuffs in the world. It is followed by fish (35%), cereals (30%), meat and dairy products (20%). Fruits and vegetables, plus roots and tubers, have the highest wastage rates of any food. In order to extend the storage period of food products, it is important to expand cold storage establishments and to increase incentives in this regard. In addition to the advantages of cold storage, the biggest obstacle we face in expanding its use is the installation, operation, and maintenance costs of cold storage. In particular, the energy consumption originating from the cooling units used in cold stores increases the operating costs considerably. Research has shown that cooling systems constitute 44.1% of the total electrical energy consumption of the factory (Carrasco et al., 2021). Energy savings can be up to 50% with improvements made in non-optimized conventional refrigeration plants. Efficient system design is the primary priority within the scope of efforts to save energy in cold stores. Since the highest energy consumption in the cooling system is on the compressor side, the studies here are focused on compressor efficiency. Variable operating conditions must be considered to reduce energy consumption from compressors. It is stated in the literature that up to 31% savings can be achieved with compressor staging (multiple compressor systems) and no-load operation strategies with correct capacity control (Carrasco et al., 2021). With appropriate capacity grading, annual system energy use can be reduced by 5–15%. In the researches conducted for the power consumption depending on the compression ratio, it is seen that by reducing the compression ratio (P_0/P_1) from 5 to 3, the power consumption in the compressor can be reduced by about 30% (Larsen and Thybo, 2004). In the study of Manske et al. (2000), efficiency comparison at part load was examined for different compressor types and it was seen that screw compressors had poor part load performance. On the evaporator side, it is necessary to use the most efficient motors possible for the application. VFD (Variable Frequency Drives) control of fans in cold stores is another valuable source of power savings, providing system energy savings of up to 2% per year. Developments in fan design and engine efficiency in recent years offer significant advantages over old-style cooling systems and cold stores. Studies on the amount of power savings relative to cooling load due to the installation of variable speed drives in evaporator fans show that savings are greater at low cooling loads (NSW Office of Environment and Heritage, 2017). Another problem is that icing on the evaporator coils will reduce energy efficiency. Actively managing defrost frequency and duration can reduce annual system energy use by approximately 3% (U.S. Environmental Protection Agency, 2020). On the condenser side, if the system demand is not met, it will cause an increase in energy. The condenser that is not maintained will create a temperature increase. This will cause operation at high temperatures. Compressors will work more; energy loss will increase. For every 1°C increase in condensing temperature, the compressor uses between 2 and 4% more energy. Air cooled condenser is preferred for cooling systems with low cooling load. Water-cooled and evaporative condensers are suitable for systems with high cooling load and low condensing temperature. Choosing the right product will increase the system efficiency (Carbontrust, 2019). One of the most important problems experienced on the refrigerant side is leakage. The reduced fluid in the cooling system will overwork the compressor, cause energy loss and harm the environment. Apart from leakage, another important issue is charging the correct amount of fluid into the system. The relationship between performance loss (capacity and efficiency) is very difficult to predict, but results from experimental measurements taken by Kim and Braun (2010), can show that annual operating costs increase by 10% when the effective refrigerant charge is reduced to 85% of the correct amount. This annual operating cost loss increases non-linearly so that at 60% correct charging the operating cost loss is +45%. As another gain point, discharge temperatures in most cooling systems are quite high (in the range of 70°C to 100°C). Therefore, the heat obtained from it can be used to heat the domestic water to about 60°C. The amount of heat recovered will be between 10 and 15% of the total heat dissipated in the condenser (Reindl and Jekel, 2007; Kassai, 2019; González and Gordín, 2020). In addition to all these, it has been observed that phase change materials, which have been researched extensively in recent years, also create energy savings during their use in cold storage (Yilmaz et al., 2020; Ismail et al., 2022; Le et al., 2023).

The aim of this study is to draw attention to the issue by analyzing the reasons that increase energy consumption in cold storages. Because the function and importance of the cold chain is increasing day by day in terms of reducing product losses and increasing product quality. Reducing energy consumption by improving cold storages will increase the quality of the stored product and reduce operating costs. It is known that in developing countries, up to 30% of food and vegetables become unusable due to various incorrect and incomplete practices in stages such as harvest, processing, storage, transportation, distribution, and consumption, and this places a serious burden on the economy (Carbontrust, 2018). For this reason, it is important to control the improvements that need to be made to reduce energy consumption in cooling systems and to increase the regulations in this regard.

In this study, research was conducted on the energy consumption of different cold storages installed in various countries. It is not possible to obtain detailed data on the general situation of existing cold stores, especially in developing countries. Cold storage operators and project companies do not share this information for confidentiality reasons. In order to determine the current situation, data collection should be carried out more by researchers. For the study, the operators of cold storages established in different countries and companies that make experienced cooling system projects were interviewed and the data were collected and analyzed. The energy consumption of the systems was examined depending on many parameters such as problems arising during the operation of the warehouses, lack of maintenance, the climate of the region where it is installed, and the electricity prices in the country where it is installed. The data obtained from the field were examined and experiments were carried out in a laboratory test room to see the behavior of a system operated in ideal conditions, and these experiments were repeated by creating inappropriate operating conditions detected in the field. By comparing both cases, energy consumption differences are examined.

2. Materials and methods

2.1. Data collection method from facilities

In order to collect data from facilities operating cold store, nearly 300 investors and refrigeration companies were interviewed. However, only 67 companies allowed data collection. Obtaining this data from companies has not been easy due to confidentiality reasons. For this reason, this data collection study took approximately 2 years. The data collected for the study are shown in Table 1. These data were collected through verbal interviews, and in case of visible differences in energy consumption, more detailed information about the maintenance and operating status was sought.

2.2. Data collected from facilities

The data of the research was obtained from 16 different countries. Some of these countries are located in the temperate climate zone, and some are in the hot climate zone. Different climate impacts were taken into account when conducting research. All of the products stored in the facilities where the research was conducted are vegetables and fruits. Meat and processed meat products, poultry products, milk and dairy products, and pharmaceutical storage facilities are excluded from this study. The storage temperature of the products is in two different types: cold (0/+4°C) and frozen storage (−18°C). Warehouse volumes vary between 170 m^3 and 18,000 m^3. The cooling capacities of the refrigeration system vary between approximately 10 hp. and 1,000 hp. R404a is generally used as a refrigerant, and in a few facilities, Ammonia, 449A and 407C gases are also used. Approximately 40% of these facilities are new facilities, while other facilities are 1 to 10 years old. Detailed information about the warehouses is shown in Table 2.

Facilities generally vary between 1 and 23 cold rooms, and if there is more than one room, the rooms are connected to the central cooling system. Cold rooms open to a common corridor, and in most applications, the corridors are also cooled in a controlled manner. Most facilities have a front-loading area, and goods are loaded into these front rooms by trucks. Figure 1 shows a schematic view of an example facility. The number of evaporators is placed as one or more depending on the size of the rooms. Figure 2 shows images from the cold stores where the research was conducted. These photographs were used to give an idea about the structure of the cold stores used in the study.

TABLE 1 Data collected from facilities.

Country/city where the cold store was established
Stored product
Cold store temperature
Cold store volume (m^3)
Number of cold stores
Capacity of cold stores (tonnes)
Capacity of the refrigeration system (kW)
Refrigerant
Average monthly electricity consumption
Refrigeration system specifications

2.3. Tests and measurements

In the second part of the study, in order to verify all this information obtained from the field; energy consumption data from a cold room operating in ideal condition was compared with the data of a cold room that was not operated properly. Energy consumption measurements were made in an empty test room with a volume of 25 m^3 in the laboratory environment. The test room polyurethane panel thickness is 10 cm, fully insulated and established in accordance with (ISO 5149-2:2014) standards. The test room is maintained regularly and all measuring instruments in the room are calibrated. The defrost system is controlled electronically and ice formation on the evaporator surface is not allowed. Entes® Network Analyzer, Televis® remote monitoring system was used for measurement and recording of test data. In Table 3, the characteristics of the measuring instruments used in the experiment are given.

The refrigeration system cooling capacity 39.3 kW for 0°C evaporation temperature / 40°C condensation temperature conditions, 16.7 kW for-18°C evaporation temperature / 40°C condensation temperature conditions. The refrigeration system was tested for 24-h performance and energy consumption first in cold room conditions (0°C) and then in freezing room conditions (−18°C). During the tests, the outdoor temperature was measured to be approximately 23°C. The refrigeration system operating differential value is set to 2°C. Ice on the evaporator surface is melted by using heating element which is a

TABLE 2 Distribution of cold storage enterprises in the research area by country.

Country	Number of cold stores	Cold storage volume (m^3)	Refrigerant	Compressor type
Turkey	12	170–18,000	404A	Semi-hermetic reciprocating
Jordan	2	209/5568	404A	Semi-hermetic reciprocating
Azerbaijan	24	197–20,160	404A	Screw/reciprocating (hermetic/semi-hermetic)
Kazakhstan	1	943	404A	Screw/reciprocating
Georgia	1	432	404A	Semi-hermetic reciprocating
Uzbekistan	3	281/1734/18090	404A/407C	Screw/reciprocating
Nigeria	4	618–1,246	404A	Semi-hermetic reciprocating
Casablanca/Morocco	4	987–5,845	404A	Semi-hermetic reciprocating
Bamako/Mali	1	936	404A	Semi-hermetic reciprocating
Misurata/Libya	1	6,131	404A	Semi-hermetic reciprocating
Kenya	2	129–6,226	404A	Semi-hermetic reciprocating
Ukraine	1	9,306	404A	Semi-hermetic reciprocating
Turkmenistan	7	693–7,830	404A	Semi-hermetic reciprocating
Ghana	1	16,401	Ammonia	Semi-hermetic reciprocating
Uganda	1	2,364	404A	Semi-hermetic reciprocating
Algeria	2	2081/10000	449A	Semi-hermetic reciprocating

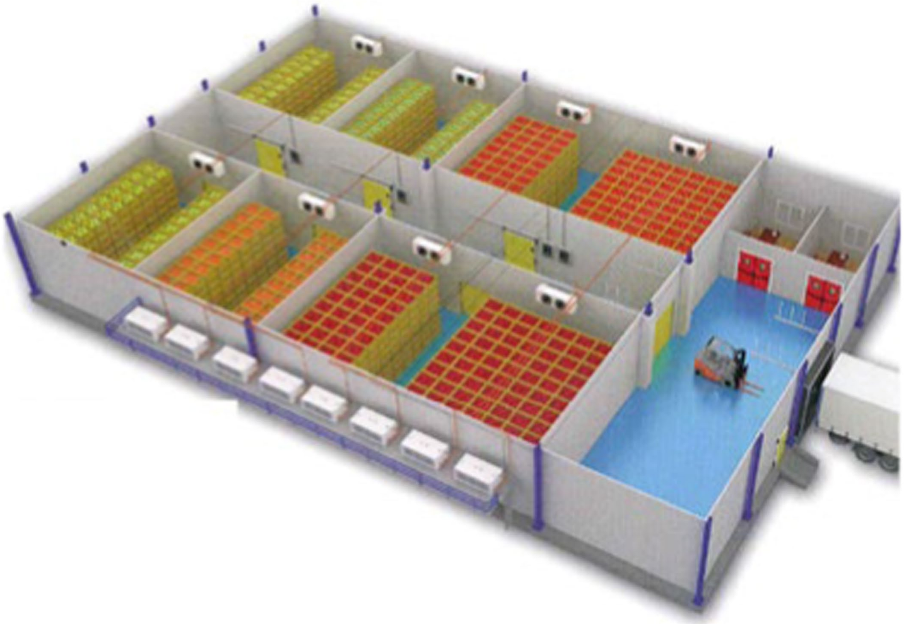

FIGURE 1
Schematic view of a sample warehouse.

FIGURE 2
Images from research facilities.

widely used defrost method in the market. The defrost system was activated every 4 h, causing short-term temperature increases in the room.

Ideal operating conditions were examined in the first configuration and in the second configuration, inappropriate operating conditions were examined to verify the data obtained from the field. During these tests, defrost cycle periods were changed to allow icing on the evaporator surface, and proper air flow was prevented by placing an obstacle in front of the evaporator. The same measurements were made for both configurations and the energy consumption of the refrigeration system depending on the operating times was compared.

TABLE 3 The measurement devices and their precisions.

Measured Variable	Measurement Device	Operation Range	Accuracy
Temperature (°C)	PT-1000	−70.0 to +240.0	±0.3% of reading
Pressure (bar)	Ratiometric pressure sensor	−1.0 to159.0	±0.5% of reading
Refrigerant mass flow rate (kg/s)	Coriolis mass flow meter	0.00 to 0.18	±0.1% of reading
Power consumption (kW)	Network analyzer	0.0 to 99.0	±0.5% of reading
Cabinet air temperature	NTC	−50.0 to +150.0	±0.3% of reading

Experimental results are used to calculate the percentages of running time and energy saving by using Eqs 1, 2, respectively (ASHRAE Handbook – Refrigeration, 2022). By measuring the compressor on–off time, the running time per cycle is calculated as follows:

$$Running\ time\,(\%) = \frac{t_{on}}{t_{on}+t_{off}}x100 \quad (1)$$

$$Energy\ sving\,(\%) = \frac{\dot{W}_{C1}-\dot{W}_{C2}}{\dot{W}_{C2}}x100 \quad (2)$$

where t_{on}, t_{off}, $\dot{W}_{C1}$ and $\dot{W}_{C2}$ represent compressor on time, compressor off time, total power consumption for config. 1 and config. 2, respectively.

The compressor power consumption $\dot{W}(t)$ is calculated by integrating the measured power for a duration t. For a 24 h energy consumption is calculated by Eq. (3).

$$W(t) = \int_{t=0}^{t=24h} \dot{W}(t)\,dt \quad (3)$$

3. Results and discussion

As a result of the research, the desired data could be obtained from 67 cold storages. These data were examined in detail and the parameters affecting energy consumption were evaluated and those with the highest impact were selected and investigated. The first of these is the climatic characteristics of the cities where data are collected. The climates where cold storages are located are divided into two different groups, hot and temperate, according to annual average meteorological data. The average summer temperature in hot climates is approximately 45°C, and in temperate climates the average summer temperature is 30°C. In order to compare energy consumption based on climate data, 25 cold storages of similar ages and characteristics operating under the same conditions were selected. 12 of these cold storages were established in the temperate climate region, and 13 of them were established in the hot climate region. All cold stores operate under cold storage conditions (range 0–4°C). The data are compared in Figure 3 by plotting the changes in the energy consumption of the refrigeration system depending on the volume of the cold store. Accordingly, while the difference in daily energy consumption of the refrigeration system is around 9% in small volumes, this difference increases to around 15% as the volume increases. The most important reason for this is that the refrigeration system works approximately 12–14 h a day in temperate climate regions and approximately 18–20 h a day in hot climate regions. It

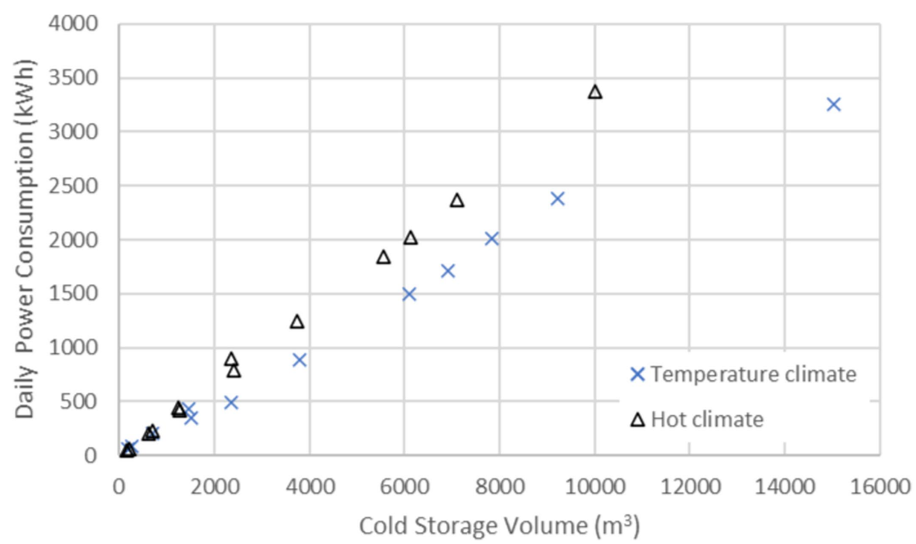

FIGURE 3
Daily power consumption-Cold storage volume relation for hot vs temperate climates.

was observed that this period increased depending on the operating conditions.

It is seen from the results that; sustainable cooling strategies need to be implemented in hot and humid climates. At this point, solar-powered cooling systems come to the fore and represent the best alternative in solving both energy consumption and environmental pollution problems. Additionally, there is a direct relationship between the availability of solar radiation and the peak of cooling demand, making solar energy a focus of great interest. In fact, solar driven adsorption cooling systems appear to be a good solution for hot climate regions. Research shows that; adsorption cooling systems can be operated with a lower heat source temperature range, are environmentally friendly as there is no ozone depletion potential or global warming potential, are more robust, less complex, and have no crystallization issues compared to absorption cooling systems (Wang and Oliveira, 2006; Wang et al., 2009; Rahman et al., 2021). Therefore, solar driven adsorption cooling systems represents the best choice to meet international guidelines in solving both energy and environmental impacts in cold storage applications (Mostafa et al., 2022).

Another issue that should be taken into consideration in hot climate regions is that the increase in condenser temperature depending on the environmental temperature will reduce the cooling capacity of the cycle due to the decrease in the liquid content in the evaporator. In other words, the high pressure and temperature of the air-cooled condenser will significantly reduce the performance of the cooling system. In this case, performance can be increased with some improvements on the condenser side. Some of those; the air-side modifications include adding spray water above condenser, wet pad before condenser, and water vapor nozzle in the condenser air flow. Studies on adding spray water above condenser, one of these solutions, have shown that it is an important tool for increasing cooling capacity and also reducing power consumption, especially in hot climates (Vrachopoulos et al., 2007; Alhamdo et al., 2015).

Another important parameter that causes an increase in energy consumption is operational conditions. Systems operated under incorrect operational conditions have been frequently encountered in the field. Problems arising from reasons such as lack of training of the operating personnel, economic reasons, and lack of quality control have a significant impact on energy consumption and product loss in cold storages. The most common operational fault is incorrect product stacking. Figure 4 shows photographs of incorrectly stacked warehouse examples. As seen in the photo, the air flow was disrupted by covering the front of the evaporator with cases, and some cases were placed in areas of the room with no air flow. There should be no obstacles that would disrupt the air flow in front of the evaporator while stacking. The placement of the crates must be done very carefully so that all the products inside the crates can cool homogeneously. Otherwise, some of the products cool down while others do not cool down enough.

Apart from stacking, there are other parameters that affect operational conditions. In order for cold storage to be carried out correctly, the necessary conditions must be defined correctly. These conditions are temperature and relative humidity values in the cold room specified in the standards depending on the product, condensation temperature, the amount of product in the cold store must be suitable for the capacity of the cold store, correct project design of processes and correct product selection, etc.

In addition to energy consumption in systems working under incorrect operating conditions, deterioration will occur because the products are not stored at the correct temperatures, which will trigger the formation of bacteria in the products and cause bad odors. For example, research has shown that; Increasing the temperature in the apple warehouse by 1°C causes a 13% loss in storage life. In interviews with operators working in facilities that are not properly operated, it has been confirmed that product losses remain above average values. However, since verifiable data could not be obtained, this subject was not included in the scope of the study.

In the research, an increase of 30 to 50 percent in energy consumption was measured in 11 facilities due to faulty operating conditions. Cold stores working under incorrect operating conditions were examined separately and comparisons were made in terms of energy consumption with cold stores operating ideal conditions in similar volumes. In Figure 5, cold stores under ideal and faulty operating conditions are compared in terms of volume-dependent energy consumption. As seen in Figure 5, with the increase in volume of the cold storage, the increase in energy consumption reaches 50% due to faulty operating conditions.

It has been shown that in some warehouses where energy consumption data is higher than other systems of similar capacity,

FIGURE 4
Typical images of stacking fault.

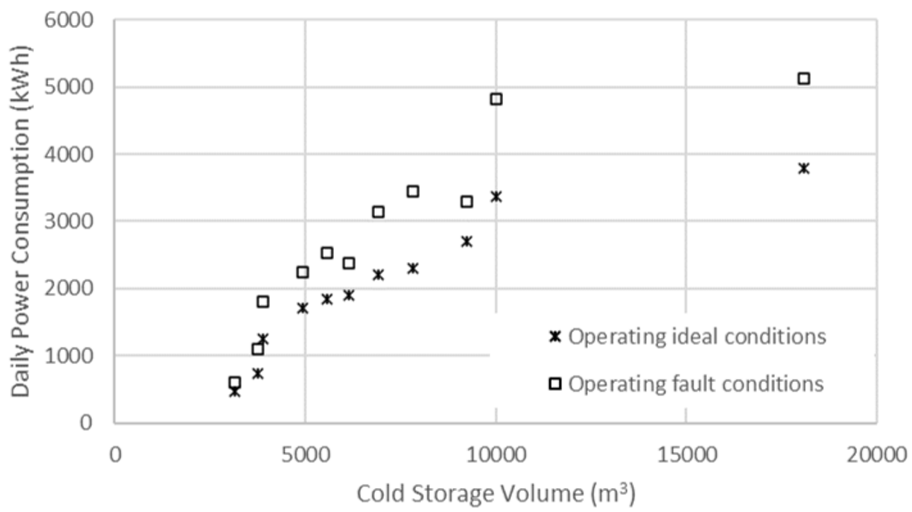

FIGURE 5
Variation of daily power consumption depending on volume for ideal and faulty operating conditions.

FIGURE 6
Images from facilities with snow formation on the evaporator surface.

the defrost cycle is not implemented correctly and the necessary air circulation between the evaporator fins is not provided due to frost. Figure 6 shows snow-covered evaporator images from some facilities.

It has been determined that in warehouses where the defrost process is not applied correctly, up to 30 percent more energy is consumed than the energy consumption expected under ideal conditions. Figure 7 clearly shows this difference in energy consumption compared to cold stores with approximately the same volume operating in ideal conditions.

Another striking point is that when an evaluation is made between the countries where data are collected, there are country-by-country differences in hourly electricity consumption prices. This affects energy expenditures depending on the installation of the same capacity cold storage in different countries. In this study, an analysis was made on this subject. Among the countries from which data were obtained, 15 countries were selected and the electricity costs in these countries were calculated by considering a cold store with a capacity of 5,000 tons. In Figure 8, the difference in energy costs when a cold storage with the same capacity is operated in different countries is analyzed. When the graph is examined, if we consider the country with the lowest electricity prices (Algeria) and the world average, it is seen that cold store operating costs increased approximately 5.5 times depending on the country. This shows that, in addition to operating conditions, the country where the cold storage is installed is an important parameter in terms of energy costs depending on electricity prices.

In countries where electricity prices are high, applying some methods used to reduce energy consumption provides savings. The role of the cold thermal energy storage (CTES) system, one of these methods, has become even more important in the last decade (Rismanchi et al., 2012). In general, CTES systems are designed to store cold energy in thermal reservoirs for use when needed. The most common media for cold storage are eutectic salt phase change

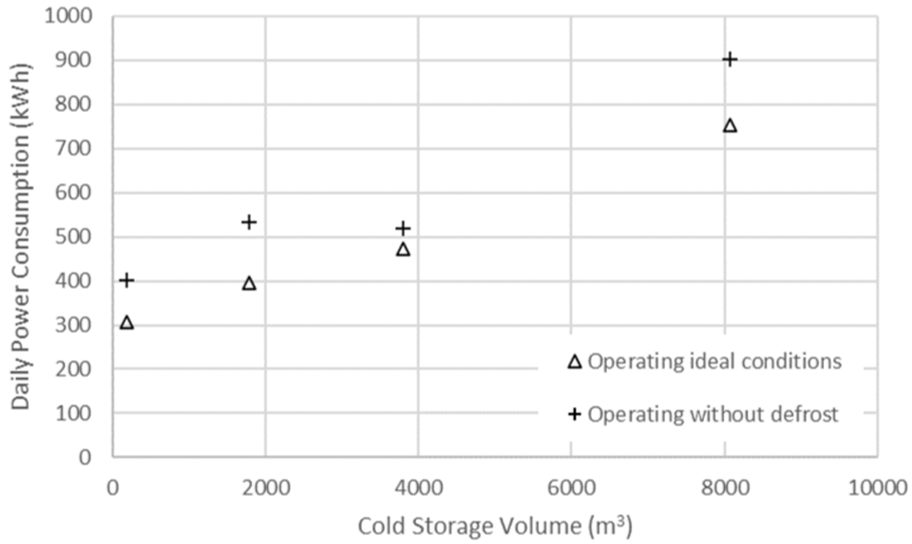

FIGURE 7
Volume-dependent variation of the effect of snow formation on the evaporator surface on daily power consumption.

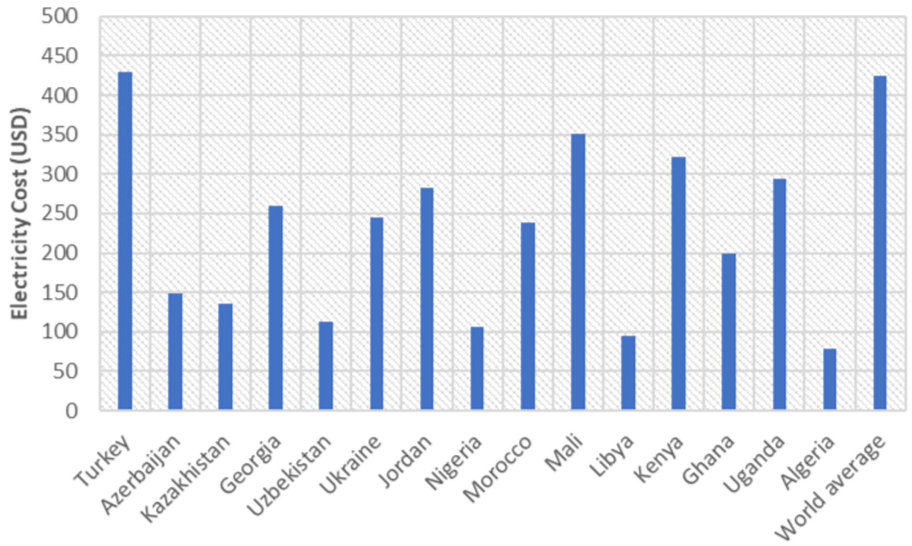

FIGURE 8
Electricity costs depending on the countries for a 5000 tons capacity cold storage plant.

materials (PCM), ice, chilled water or paraffin, depending on the application and temperature ranges (Al-Rabghi and Akyurt, 2004; Bahnfleth and Song, 2005). This method has the benefit of reducing energy usage by shifting electricity consumption to off-peak hours when the electricity price is generally cheaper.

In order to verify all these data, the energy consumption of the cold store operating ideal conditions (config. 1) and the cold store with faulty operating conditions (config. 2) were examined in the test room established. Ideal operating conditions were examined in the first configuration, and as seen from the graphics, the test chamber reached 0°C in approximately 20 min (Figure 9A) and-18°C in approximately 1 h (Figure 9B). As a result of the energy consumption measurements made with the network analyzer during the experiments, it was recorded that the total energy consumption of the freezing room was 69 kWh, and the cold room was 26 kWh. This consumption data includes compressors, condenser fans, solenoid valve on the device and electrical components. Evaporator fans, defrost resistors, remote control panel and evaporator solenoids are not included. Using the results obtained from the measurements for both configurations; running time, energy saving and 24 h energy consumption was calculated with Eqs 1–3. The results of the calculations are shown in Table 4. According to the calculations, in the 2nd configuration, the operating time of the compressor increased from 3.2 h to 4.1 h, and the energy saving is 32.2% when the system operates under ideal conditions. Similar studies in the literature report that energy

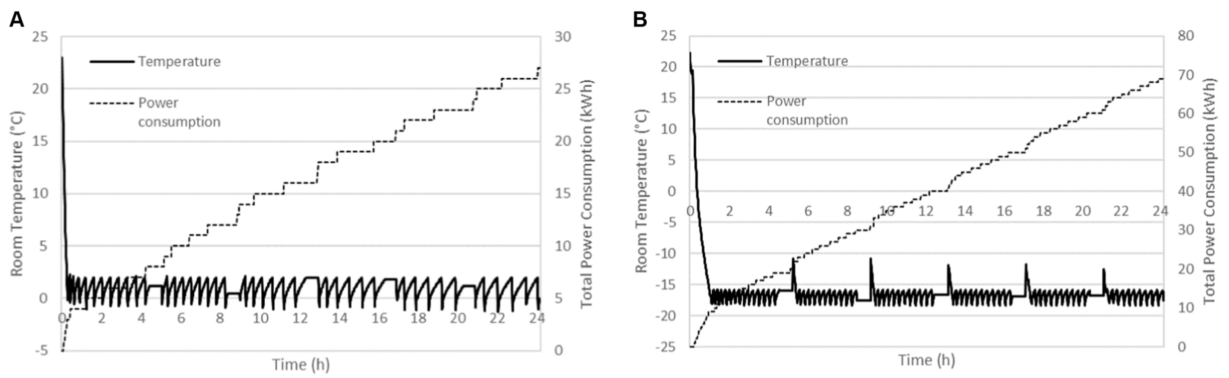

FIGURE 9
Time dependent variation of 399 room temperature and total power consumption for Cold room **(A)**, and Freezing room **(B)**.

TABLE 4 Energy consumption of the cold room for two configurations.

	Config. 1	Config. 2
Total compressor on time in 24 h	3.2 h	4.1 h
Total compressor off time in 24 h	20.8 h	19.9 h
Running time (%)	13.3	17
Energy saving (%)	−32.2	–

savings in non-optimized conventional facilities can reach up to 50% (Industrial Refrigeration Guide, 2017). It is clear that this value will increase as operating conditions worsen. For this reason, the importance of correct operation of the system as well as the design stages should be emphasized.

4. Conclusion

The first waves of the global climate crisis began to spread to Anatolia. The farmer is aware of the change in air, soil, and yield. Agricultural reports contain dramatic results, reminding decision makers, researchers, and citizens of our responsibilities. In the latest report of the Intergovernmental Panel on Climate Change (IPCC), it is predicted that temperature increases will be around 2.5–3 degrees for 2050 and will reach 6 degrees by the end of this century. Today, while the observed effects of a 1 degree increase in temperature are so great, the economic, social, and environmental risks posed by a 6 degree increase show that climate change is one of the biggest risks faced by human history. While all this is happening, most of the agricultural products produced are wasted because they cannot be stored correctly. Therefore, cold storage emerges as a much more important player. Incentives for the expansion of cold storage are increasing day by day. However, here too, as a major handicap, the energy increases consumed by cold stores that are not operated properly.

In this study, data were collected from cold stores established in developing countries. When these data are analyzed, it is seen that the increase in energy consumption caused by improperly operated warehouses reaches almost 32%. This value can be reduced by controlling it with correct operating methods and increasing regulations. Apart from this, in order to reduce the increase in energy consumption due to uncontrollable ambient temperature and country policies, new technologies must be integrated during the cold storage installation phase and these disadvantages must be eliminated with correct project design. Increasing the data collected from existing cold stores is very important in terms of drawing attention to this issue. For this reason, future studies and research in different countries will make the subject more clear. In addition, the measurable determination of product losses as well as energy loss in improperly operated cold stores, should be the subject of future studies.

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

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

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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A secure food supply chain solution: blockchain and IoT-enabled container to enhance the efficiency of shipment for strawberry supply chain

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The supply chain systems in the food industry are complex, including manufacturers, dealers, and customers located in different areas. Currently, there is a lack of transparency in the distribution and transaction processes of online food trade. The global food supply chain industry has enormous hurdles because of this problem, as well as a lack of trust among individuals in the sector and a reluctance to share information. This study aims to develop a blockchain-based strawberry supply chain (SSC) framework to create a transparent and secure system for tracking the movement of strawberries from the farm to the consumer. Using Ethereum smart contracts, the proposed solution monitors participant interactions, triggers events, and logs transactions to promote transparency and informed decision-making. The smart contracts also govern interactions between vendors and consumers, such as monitoring the status of Internet of Things (IoT) containers for food supply chains and notifying consumers. The proposed framework can be extended to other supply chain industries in the future to increase transparency and immutability.

KEYWORDS

strawberry supply chain management, smart contract, Ethereum blockchain, Internet of Things, food supply chain

1 Introduction

From the agricultural area to the consumer's table, the food supply chain (FSC) describes how food is grown, processed, refined, transported, sold, consumed, and discarded. It involves interdependent activities and parties, such as producers, manufacturers, distributors, retailers, and consumers, as shown in Figure 1. To ensure the availability of goods, efficient supply chain management is needed for reliability and food safety for consumers, enhancing the efficiency and sustainability of the food system (Kamble et al., 2020). In developing countries, the supply chain for food faces numerous obstacles. These include the need for trust among stakeholders, which is contingent upon the demands of the end users for credibility and traceability. Insufficient or missing information can make it difficult to manage risks, delays, or disruptions in supply chain operations (Kittipanya-Ngam and Tan, 2020). However, blockchain technology is recognized as one of the most effective solutions to tackle these challenges (Niu and Li, 2019).

The food systems are complicated and continually evolving. These days, consumers place a greater emphasis on food safety, quality, and nutrition than on quantity alone (Yakovleva, 2007; Manning, 2018). Given the hazards associated with food spoilage and potential harm, the FSC is essential in assuring the safety of products. As a result, improving traceability is becoming increasingly important in the agriculture sector. The safety, nutritional value, and quality of agricultural foods and goods, which are extremely susceptible to these dangers, are of particular concern to consumers. It is crucial that supply chain authorities provide information promptly and properly if they want to win the trust of end users. Consumers are particularly concerned about the safety, nutritional value, and quality of agricultural foods and products, which are highly susceptible to these risks (Dabbene and Gay, 2011).

For supply chain authorities to acquire the trust of end users, it is essential that they deliver information quickly and accurately. Strategies for ensuring food security place equal emphasis on food quality and quantity. Since organic farming methods are expected to produce food with greater nutritional content and higher quality than traditional farming systems, the relevance of food quality and safety is rising day by day in these systems. To sustain life and advance good health, it is essential to have access to adequate quantities of safe and nourishing food. Furthermore, it is crucial to maintain adherence to standards of quality, integrity, and credibility throughout the supply chain process. Several regulatory agencies have implemented standards to improve transparency, security, and quality of the supply chain traceability system bar codes for identifying the origin of products (Turri et al., 2017). The Chinese Government employs a comparable enforcement system. These regulations seek to increase the transparency of the product tracing system and guarantee high product quality (Mastilović et al., 2023).

Additionally, more cutting-edge technologies are used in modern supply chain management (SCM) to give businesses more flexibility. Integrations aid businesses in protecting their products while facilitating the movement of commodities backward. SCM also highlights the importance of creating alliances, partnerships, and collaborations. Modern food supply channels have the potential to be revolutionized by blockchain technology (Kouhizadeh and Sarkis, 2018; Queiroz et al., 2020). Transactions between peers are supported in this decentralized system, which also validates transactions by eliminating middlemen. Scholars have looked into how the use of blockchain technology might improve supply chain efficiency and address issues such as information inequity and decency in food recalls. Researchers have investigated how the utilization of blockchain technology contributes to the enhancement of supply chain performance and resolves problems such as information inequality and food recall indecency (Zhao G. et al., 2019). Blockchain technology enables users and suppliers to instantaneously check transaction information by gathering data on all transactions within a given timeframe. This results in the creation of a digital record for verification and confirmation. Currently, a trustworthy blockchain-powered traceability system for fruits and vegetables distributes necessary traceability information along the supply chain. Furthermore, a reputation-driven smart contract was established to stimulate network nodes to contribute to traceability data. In a recent study, the authors evaluated and executed the system, determining that it increases query efficiency, safeguards personal data, guarantees the integrity and trustworthiness of supply chain data, and fulfills the necessities of relevant applications (Rünzel et al., 2021).

The application of IoT technology in the FSC has enabled real-time monitoring, data acquisition, and data transfer capabilities (Coronado Mondragon et al., 2021), while blockchain technology

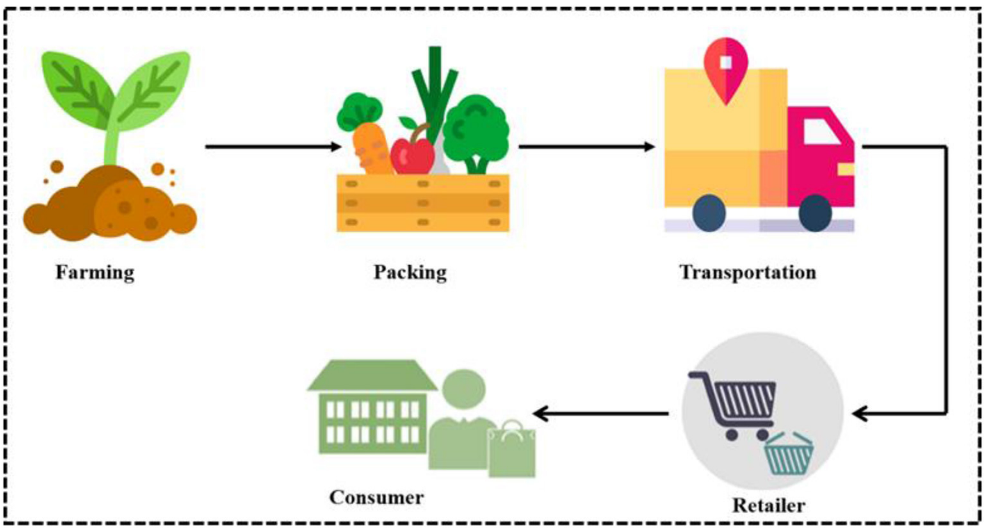

FIGURE 1
Traditional Strawberry Food Supply Chain (SFSC).

provides improved dependability, product/service security, and traceability (Behnke and Janssen, 2020). The integration of IoT and BLC can provide FSC with simultaneous capabilities for data security, trust, visibility, dependability, and real-time data sharing (Lezoche et al., 2020). Implementing blockchain and IoT in agriculture can facilitate the monitoring and tracing of various aspects to guarantee the safety and quality of food products. The development of an FSC tracking system for halal livestock supply chains utilizing hazard analysis and critical control points (HACCP), blockchain-based technology, and IoT devices is one example. A supply chain is made up of all the steps used to deliver a finished good or service to the customer. The process may involve collecting raw materials, delivering them to the production site, and then transferring the final goods to a warehouse or retail location where they may be distributed to the customer. This system collects data from IoT devices at various stages of the supply chain and archives it securely on a blockchain-based platform for immutable record-keeping. In addition, the study examines the implementation of the system and evaluates its efficacy in enhancing supply chain transparency and quality control (Rejeb, 2018).

The primary advantage of utilizing blockchain technology during a pandemic is that it eliminates trust issues among all supply chain participants. This is achieved by sequentially documenting all transactions on the blockchain and broadcasting the events to all authorized parties, including consumers. Many researchers have demonstrated FSC and blockchain combinations with developing technologies, such as the Internet of Things, the cloud, smart contracts (Srivastava and Wood, 2015), big data, analysis through case studies (Verhoeven et al., 2018), and survey techniques (Hackius and Petersen, 2017). These methods have resulted in significant developments, such as enhanced traceability effectiveness, supply chain transparency for users, and specific challenges, such as scalability restrictions, emerging technologies, and the absence of regulatory frameworks (Wang et al., 2019).

It is crucial that supply chain authorities provide information promptly and properly if they want to win the trust of end users. The main goal of this research is to address the strawberry supply chain (SSC)-related issues and create a blockchain-based management system for secure FSC that can track and manage data for each supply chain node. Second, research on how IoT and blockchain might enhance supply chain management to have a better grasp of information management systems. Blockchain-based SSC management system incorporates blockchain technology and Internet of Things-enabled smart containers. Smart containers can transmit precise sensory data to cloud storage, enabling efficient supply chain management. Moreover, if any violation occurs, the containers can send out notifications that are recorded on the blockchain ledger. Furthermore, the objective of the study is to assist other supply chains in addressing their management and safety supervision concerns. This inquiry seeks to provide answers to the following research queries.

RQ1: What are the various challenges of blockchain adoption in FSC?

RQ2: How can IoT-enabled containers facilitate the transportation of food from its source to its destination?

RQ3: How does blockchain provide food traceability and end-to-end visibility?

- An innovative blockchain-based solution is introduced for all participants in the supply chain that eliminates the need for third-party involvement.
- A novel solution is presented for the IoT-enabled container transportation of FSC products from the source to the destination. This solution involves detecting any violations within the supply chain and logging the corresponding events using a smart contract.
- The proposed solution ensures transparency and provides end-to-end visibility of the product from its source to the destination.

For the FSC sector, we suggested a blockchain-based FSC solution. To guarantee the delivery of strawberry products of the greatest quality to end users, our suggested methodology combines IoT and blockchain technology, notably smart contracts. The ecological sustainability of the strawberry supply chain is evaluated.

The rest of the study is structured as follows.

Section 1 presents the introduction, Section 2 covers the conceptual context and literature review, and Section 3 provides a thorough explanation of the proposed methodology. In Section 4, the results and analysis are presented, followed by a discussion of the results in Section 5. Finally, Section 6 discusses the conclusion, implications, and limitations.

2 Related work

The food supply chain refers to the complex network of activities involved in producing, processing, distributing, and delivering food products from the point of origin to the end users. It encompasses various stages, including production, processing, packaging, storage, transportation, and retailing. In this section, we discuss the related work of FSC and its challenges, problems, and state-of-the-art work.

The author (Zhao X. et al., 2019) discusses a consolidated cold and modified atmosphere packaging system for fresh strawberries in supply chains. The term "supply chain performance" refers to the actions taken by the extended supply chain to meet end-user demands. This includes making sure that products are available, deliveries are made on time, and the supply chain has all the inventory and capacity it needs to do so in a timely manner. The proposed system aims to improve the shelf life and quality of strawberries during transportation and storage by reducing the rate of respiration and controlling temperature and humidity levels. The study also addresses the challenges related to supply chain management and safety supervision in the strawberry industry. The research aims to provide solutions to these issues and assist other supply chains in dealing with their management and safety concerns.

This article (Kelly et al., 2019) introduces a novel approach for evaluating the impact level of each step in the SSC on fruit quality. Throughout the whole supply chain process, strict adherence to norms of trust, honesty, and quality is essential. Several regulatory bodies have put regulations into place to increase the openness,

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safety, and quality of the supply chain traceability system. This system uses bar codes to identify the country of origin of products. The study focuses on physical, chemical, and microbial factors that affect strawberry quality during transportation and storage. By analyzing the data collected from different steps in the supply chain, the researchers developed a comprehensive evaluation framework to identify the critical stages that have the most significant impact on strawberry quality. The proposed approach can assist supply chain managers in making informed decisions to improve fruit quality and reduce waste. The author (Duan et al., 2020) presents the possibility of the blockchain system to address supply chain problems, including food fraud, food safety, and transparency. The authors identify three main themes in the literature: (1) the advantages of implementing blockchain technology in the FSC; (2) the problems for the adoption of blockchain in the food supply chain; and (3) the outlook of blockchain usage in the food supply chain. The study suggests that blockchain technology possesses the ability to offer benefits including increased transparency, trust, and safety, in addition to improved FSC tracking and accountability. Nevertheless, the authors find a number of adoption barriers, such as high expenses for implementation, regulatory ambiguity, and a lack of compatibility between blockchain systems. The authors (Kaur et al., 2022) examine the existing status of studies on the combination of blockchain and IoT within the management of food supply chains. The study highlights the potential advantages of integrating blockchain and IoT, such as increased FSC transparency, transparency, security, and efficiency. The authors identify a number of obstacles facing the food supply chain, including security of food, accountability, and transparency, and propose that blockchain and IoT can help overcome these obstacles. The authors provide a comprehensive overview of the theory of evolution, advantages, and potential applications of blockchain and IoT in the administration of the food supply chain. They indicate that more study is necessary to fully comprehend the challenges and opportunities of integrating protocol standards and the resolution of data security and privacy concerns. Using the IoT, the authors (Bhat et al., 2021) propose a solution to enhance the interoperability of blockchain networks in agricultural and FSC supervision. The authors argue that the use of blockchain-based technologies in food and agricultural supply chain management can improve supply chain transparency, traceability, and stakeholder trust. The researchers suggest that their suggested approach can contribute to the development of a more effective and transparent agriculture and FSC management system. To fully realize the potential of blockchain technology and IoT devices in supply chains and to address the difficulties of interoperability and data privacy, they propose that further research is necessary.

This study (Saurabh and Dey, 2021) explores the advantages of utilizing blockchain technology, such as increased transparency, traceability, and efficacy, the challenges associated with its adoption, such as security and confidentiality of data concerns, and the need for standardization. The authors conclude that blockchain technology has substantial potential for promoting sustainable food and agriculture supply chains. They suggest that additional research is required to resolve the challenges related to blockchain's acceptance and to develop standard protocols and frameworks that facilitate the interoperability of different blockchain networks. The study (Kumar et al., 2022) details the examination of the IoT and blockchain in the food supply chain, identifying the main adoption barriers, such as the absence of standard processes and security and confidentiality of data concerns. The authors also discuss potential solutions to these challenges, such as the implementation of standardization for automating transactions, the integration of artificial intelligence and machine learning into processes, and the collaboration between diverse stakeholders in the food supply chain. The authors argue that additional research is required to comprehend the difficulties and possibilities presented by the approach and develop standard protocols that facilitate interaction among blockchain networks. Moreover, the authors contend that cooperation among FSC stakeholders is essential for the effective execution of blockchain and IoT.

In this context (Abdallah and Nizamuddin, 2023), the current research presents blockchain technology within the pharmaceutical supply chain. Increased transparency, accountability, and efficiency are just some of the benefits discussed, and the authors also delve into the difficulties of implementing blockchain technology, such as data privacy and security concerns and the need for standardization. Data, blockchain, middleware, and applications are the four tiers of a blockchain architecture proposed by the authors. They show that this layout has the potential to be a flexible and scalable answer to the problem of incorporating blockchain technology into the pharmaceutical supply chain. Their findings imply that more investigation is required to address adoption issues and provide standardized protocols and frameworks for blockchain networks to communicate with one another. The proposed approach in Hasan et al. (2019), uses the smart contracts and the logistics industry with a more trustworthy, cost-effective, and transparent system for managing cargo procedures. The article also explores the ways in which smart contract technology might be used to improve logistics operations in areas including real-time cargo tracking, automated payment processing, and risk management. According to the authors, smart contract technology can improve logistics efficiency and security by standardizing procedures, cutting costs, and boosting confidence in the business. The authors argue that there is a significant opportunity for smart contract technology to revolutionize the logistics sector. They imply more studies are required to remove the barriers to adopting and providing standardized protocols and frameworks that can make different smart contract networks interoperable.

3 Proposed framework

Using a blockchain-based system that includes sensor data from IoT-enabled shipments and Ethereum smart contract technology is central to our proposal for enhancing the integrity of the strawberry supply chain. These crucial components in the process of triggering warnings are smart contracts that provide all parties involved with the ability to continuously monitor and trace during shipment. The sender, receiver of the shipment, an IoT-enabled container, an Ethereum network that runs the smart contract, and a cloud-based Message Queue Telemetry Transport (MQTT) server that stores, retrieves, and publishes sensor data generated by IoT sensors are some of the components that make up the proposed system. Figure 2 illustrates various components that make up the proposed system. This solution involves four major entities, namely, the sender, the receiver, the IoT device included within the shipment container, and the smart contract. The proposed approach addresses the most important design aspects of our solution, which involve four major participants. In Ethereum, one-of-a-kind Ethereum Addresses (EA) and key pairs can be generated instantly, and there is no centralized authority that is responsible for administering the distribution of keys. The sender is responsible for constructing the smart contract that contains the terms of the sale, which may include variables such as temperature, route, and payment terms.

3.1 Blockchain-based SSC

The proposed system utilizes smart contracts and blockchain to retain all transactions and data in blocks. Its implementation in the SSC can yield significant food safety benefits. Various formats for product transparency exist: Ingredients are listed clearly on the label. Safety Data Sheets for the product that are exhaustive and list all substances that are added knowingly. Attestation forms demand complete material disclosure. The principle of transparency mandates that any information aimed at the public or the data subject should be brief, publicly available, and understandable. It also mandates the use of plain language and, when applicable, visuals. This system unites diverse stakeholders in a decentralized supply chain, assuring food safety by providing a transparent and immutable ledger of transactions. With its ability to readily trace the origin of food items, blockchain technology can assist the food industry in improving overall quality and streamlining transportation. In the food sector, there are numerous manufacturers, distributors, and customers who are spread out across the supply chain systems. Industrial procedures that transform fresh produce into consumable food products are referred to as food manufacturing. It entails several procedures, including sourcing, preparing, producing, packaging, and distributing food. Many components used in the production of food are also used in the production of apparel and automobiles. In addition to enhancing food safety, implementing blockchain in FSC can also increase manufacturer and supplier accountability.

3.2 Smart contracts between trading partners

A smart contract can increase supply chain transparency and accountability, allowing consumers to readily identify and avoid unethical practices. This can ultimately result in improved

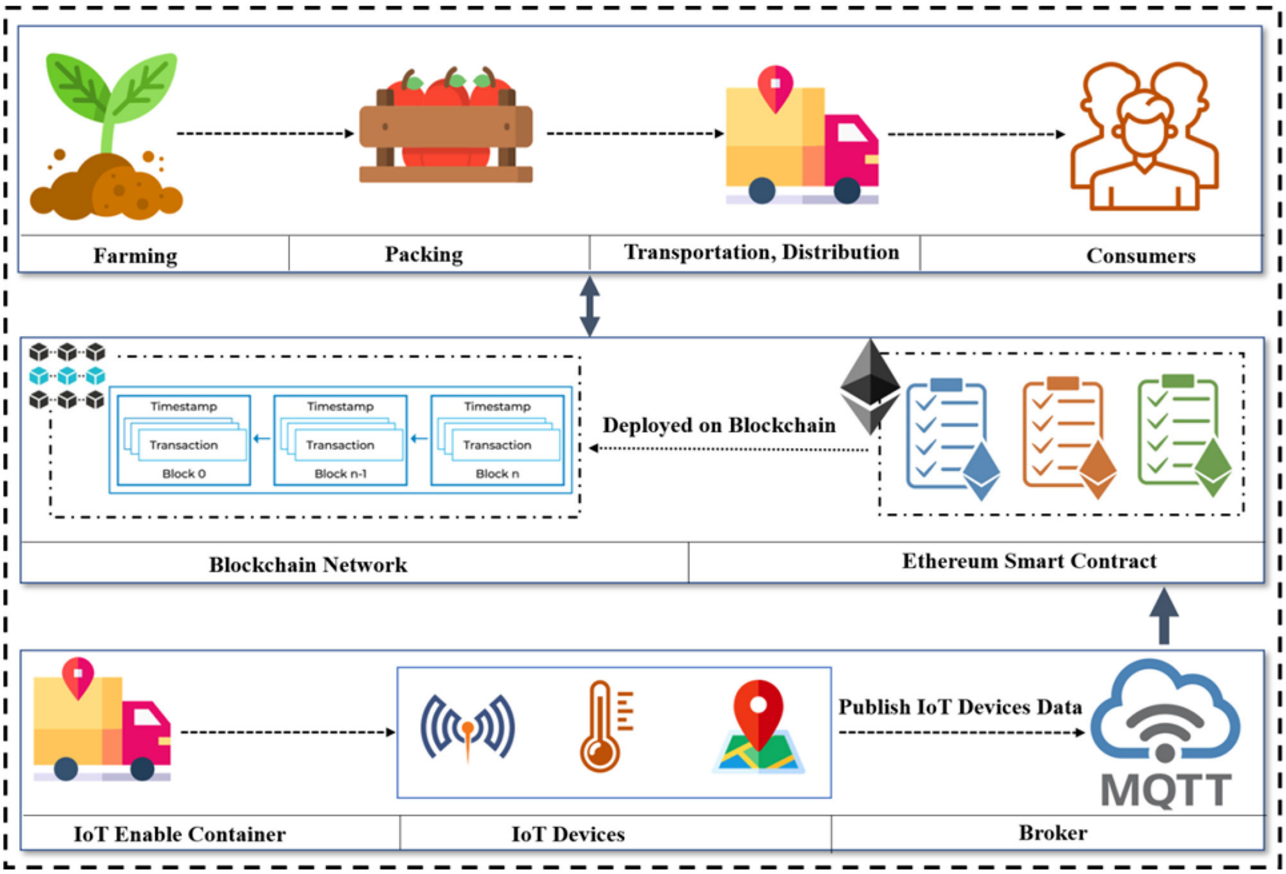

FIGURE 2
Proposed methodology of SFSC.

```
function PerformeSelfCheck(int result) OnlyContainer public
{
    require(state == packageState.checkRequirements);
    selfcheck_result = result;

    if(selfcheck_result == 1)
    {
        state = packageState.PreparedforDispatch;
        emit SelfCheckDone("Self Check result is Success");
        message = "Self Check Done Everything is Okay";
    }

    else if(selfcheck_result == 0)
    {
        state = packageState.Aborted;
        emit SelfCheckDone("Shipment Aborted: Failure , container must be fixed.");
        message = "There is Something Issue Please fix it";

    }
```

FIGURE 3
Smart contract for self-check (success scenario).

```
function sendMoney() payable OnlyReceiver public
{
        sendMoneyToAddress = msg.value;
        address to = 0x5B38Da6a701c568545dCfcB03FcB875f56beddC4;
        payable(to).transfer(sendMoneyToAddress);
        message = "Money Has Been Received";
}
```

FIGURE 4
Smart contract for deposit money.

product safety and increased profits for all involved parties. Real-time updates and a digital record of all food transactions can also facilitate traceability and enhance supply chain management processes. In the proposed system, a distinct smart contract is created for each IoT-enabled shipment container, and all entities are linked to the blockchain-based smart contract. Smart contract for self-check is shown in Figure 3, and smart contract for deposit money is shown in Figure 4.

3.2.1 Variables

Smart contract uses variables that represent numbers and states that reflect specific circumstances and variables, which can be set to private or public. The addresses of the parties involved are also saved as variables in the smart contract, along with the state of the package and the category of violation.

3.2.2 Methods

Methods are essential smart contract functions that enable the contract to carry out specific actions. The methods of the proposed smart contract are intended to generate notifications for violations. The functionality of these methods is directly related to the purpose of the contract, and they may be made public or restricted to specific parties.

3.2.3 Modifiers

Modifiers are used to introduce additional requirements or conditions to a function or method. Before executing a function, they can be used to restrict access to specific methods or verify that certain conditions are met.

3.2.4 Events

Events are a method for logging and tracking essential data within a smart contract, as well as notifying all relevant parties of significant changes or updates. In the context of the proposed system, events can be used to record and disseminate information about supply chain violations, ensuring that all relevant stakeholders are aware of potential problems in real time. This can aid in enhancing supply chain transparency and

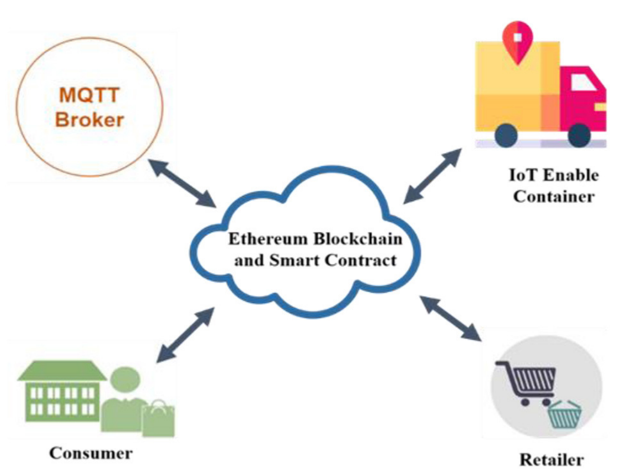

FIGURE 5
Blockchain platform for supply chain entities.

accountability, thereby helping to ensure the safety and quality of food products for consumers.

3.3 Role of supply chain nodes

Supply chain nodes play a crucial role in the movement and management of goods and information throughout the supply chain. They are the key points where various activities such as sourcing, production, storage, transportation, and distribution take place. Supply chain entities with smart contracts are shown in Figure 5.

3.3.1 Manufacture

Accepting raw materials, supervising the manufacturing process, conducting quality control checks, and administering packaging are all responsibilities of the supply chain manager. In addition, it is essential for the manager of the supply chain to ensure high levels of production, quality, and worker efficiency.

3.3.2 Supplier

The company establishes a network of contacts and employs suppliers for the necessary raw materials to manufacture its product. During this phase, supply chain administrators are responsible for maintaining company–supplier relationships. In this component, a supply chain manager is primarily responsible for sending products safely to the supply chain.

3.3.3 Container

IoT-enabled containers in the supply chain are cargo containers equipped with IoT technology. These containers are outfitted with sensors and devices that can capture and transmit real-time data such as location, temperature, humidity, pressure, and other environmental factors that impact the condition of the transported goods. The IoT platform collects and analyzes data from these sensors in order to monitor the condition of transported products. In the event of any deviations from the desirable conditions, alerts are generated so that the necessary steps can be taken to prevent any potential damage to the goods.

3.3.4 Sender

The sender initiates a smart contract which is referred to as the seller, who conducts a package inspection of the IoT container to ensure the condition of the freight. On the other hand, the consumer is responsible for the deposit payment (in Ether), and payment will only be made to the vendor if the shipment is delivered to the consumer without damage or violation.

3.3.5 Receiver

When a consumer requests a return, the company arranges for the collection of any defective, excess, or unwanted items. It is essential to have an efficient and effective return management system. If a product is damaged, the manufacturer either repairs it or disposes it in a responsible manner. Nevertheless, if a returned product is still in excellent condition, the company may decide to return it to the warehouse for resale.

3.4 Transport practices for SSC

Fresh fruits, such as strawberries, must be transported and stored with great care to preserve their quality and extend their expiration life. Due to their sensitivity to temperature, it is essential to adhere to specific transportation requirements and conditions to ensure their marketability. The proposed method is designed particularly for handling perishable fruits, and the cold chain principle is utilized to maintain their quality during transport. Strawberries are especially delicate and necessitate refrigerated transport and cautious route planning to ensure prompt and secure delivery. During the transfer of food products from warehouses to refrigerated trucks, temperature fluctuations can cause condensation on the surface of the product, resulting in premature spoilage. Refrigerated vehicles with air circulation devices are used to ensure that the containers are properly ventilated and that ethylene and heat generated by the supply chain process are removed. To confirm ventilation, the distance between the top surface of the container and the roof of the cooler must be between 30 and 50 cm, and 3 to 5 cm should be maintained between the rows of boxes. Before loading, strawberries must be chilled to 32°F. During the summer months, strawberries tend to warm up during transport, so it is essential to avoid loading damaged containers and cool them to 32°F before loading. Some food items[1] with the temperature modes during the transportation of fruits are shown in Table 1.

1 https://frutline.com/blog/usloviya-perevozki-fruktov-i-ovoshhej/

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TABLE 1 Temperature modes of different fruits.

No	Food type	Temperature, °C
1	Strawberry	−0.5 to +0
2	Black cherry	0 to +2
3	Apples	+2 to +4
4	Plums	0
5	Pears	0 to +1

3.5 IoT enable container

IoT can enable container supply chain management by providing real-time visibility, tracking, and monitoring of containers throughout their journey from the starting to the ending point. This allows for better management of the container supply chain, including improved tracking of the containers' location, temperature, humidity, and other environmental conditions. IoT devices can also provide information on container conditions, such as whether the container has been damaged or tampered with, and if it has been opened during transit. This allows for early identification of potential problems and enables the container supply chain to take corrective actions in a timely manner. Furthermore, IoT-enabled containers can provide data that can be used to optimize the supply chain and reduce costs. By tracking the location and status of containers, shipping companies can improve their delivery times, reduce fuel costs, and optimize their routes. Overall, IoT can provide a significant improvement in container supply chain management, offering better visibility, increased efficiency, and cost savings.

All sensors are linked with A Raspberry Pi 3 inside the container, which serves as the onboard IoT processing hardware. For periodic data transmission to the MQTT server and communication with the Ethereum-based blockchain network, 4G or 5G connectivity is needed. Both the sender and the destination automatically receive push warnings and notifications when specific requirements are not satisfied or violations take place while being transported. On the Raspberry Pi, a rule-based engine that requires specific rules to be specified for each sensor reading is used to find violations. The criteria and their thresholds may be altered in accordance with the nature of the cargo with the approval of the sender and receiver. Failure to comply with the rules constitutes a violation. The cargo container closely monitors the sensor data and starts particular processes when they diverge.

3.6 MQTT server

A broker is a server that facilitates communication between clients within an MQTT system. MQTT is frequently implemented in IoT-based initiatives. Using specific rules for each sensor input, the Raspberry Pi's rule-based engine identifies infractions. Any deviation from these guidelines constitutes a rule violation. All data collected by IoT sensors are installed in the container and published on an MQTT server hosted in the cloud. IoT-enabled container, cloud-hosted MQTT server, sensors, Raspberry Pi, and the contract on the Ethereum blockchain are all interconnected, as shown in Figure 6. Whenever any violation occurs, the readings are sent to the smart contract, which communicates with the seller and the consumer.

3.7 Solution for FSC industry

The SSC is susceptible to a number of industrial obstacles. As strawberries are a seasonal crop, their highest production occurs in many regions during the spring and early summer. Growers and distributors must coordinate their efforts to ensure the year-round availability of fresh strawberries, which can complicate the supply chain. In addition, strawberries are highly perishable and require cautious transportation to preserve their quality; therefore, delays or insufficient temperature control during shipping can result in spoilage or a shorter shelf life. As with any other food item, it is essential to guarantee the safety of strawberries by preventing their contamination with harmful substances. This necessitates caution on the part of growers and processors, as well as the maintenance of appropriate storage and handling procedures by distributors, in order to minimize the risk of spoilage or contamination. The FSC industry must continue to address issues such as labor, transportation, food safety, and sustainability and collaborate to ensure a reliable and sustainable strawberry supply chain. The proposed methodology intends to address these issues.

Transparency and visibility of products are improved using blockchain technology and IoT devices. IoT enables tracking of the movement of strawberry containers from the source to the destination. Each step in the supply chain can be recorded on the blockchain, and IoT devices monitor the temperature and humidity of the strawberries during transport, ensuring that they remain fresh and of high quality. MQTT server publishes data of sensors to Ethereum-based smart contract. The safety of strawberries can be ensured by utilizing blockchain technology to create an immutable record of their origin, processing, and distribution. This aids in the prompt identification of any food safety issues, and the source of the problem can be readily identified. The transactions are perpetually recorded in a distributed ledger, ensuring the immutability of the product. In addition, the journey of the container is monitored by IoT sensors that measure crucial parameters such as temperature and humidity. The optimal SSC can be achieved by integrating blockchain and IoT technology, enabling supply chain-wide transparency. The automation of specific aspects of the strawberry supply chain, such as quality control and payment processing, is also dependent on smart contracts. For instance, a smart contract could be designed to release payment to the farmer only after the strawberries have been delivered to the distributor and have passed quality control. Overall, blockchain technology and IoT devices present various potential solutions to the supply chain issues encountered by the strawberry industry. These technologies can enhance traceability, food safety, sustainability, and automation, assuring consumers of a reliable, high-quality supply of fresh strawberries.

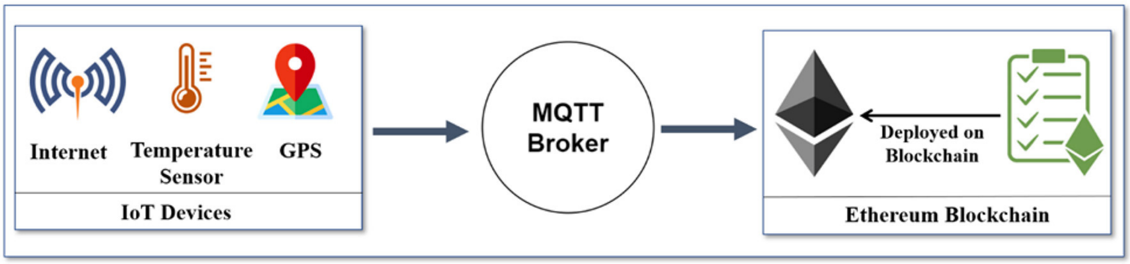

FIGURE 6
Interaction of MQTT server with IoT and blockchain.

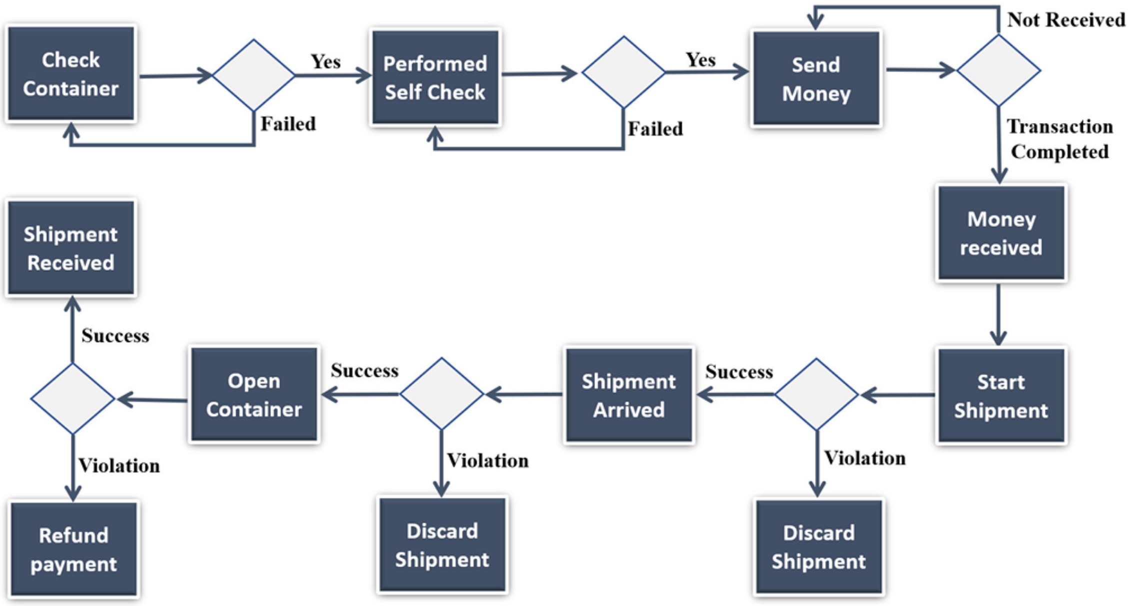

FIGURE 7
Complete flowchart of success scenario SFSC.

4 Implementation details

The Solidity code for the smart contract was created using the online Remix IDE. Three parties are involved in a contract, namely, the owner of the contract, the IoT-enabled container, and the receiver. All of these parties have a unique Ethereum address and can interact with the contract in different ways at different times. The smart contract was created with modifiers that limit access to particular supply chain processes, preventing any of the parties from calling unauthorized functions. A complete success scenario of the proposed system that is provided for the stakeholders is discussed in Figure 7.

For instance, checkContainer () and checkRequirements () can only be accessed by the sender, and all supply chain nodes are linked to the smart contract. The blockchain network has assigned each node a distinct EA. IoT makes it possible for containers to attach various sensors to the Raspberry Pi and for the MQTT server to continuously harvest sensor data and send it to the smart contract. For IoT devices to communicate with one another via the MQTT protocol, an MQTT server serves as a central hub. It controls the message flow between devices, ensuring that only the intended receivers receive messages. A cloud-based server of MQTT has a communication protocol for smart contracts and sensors. The contract will first be available on a blockchain network; only the owner can make it available.

By employing modifiers, different roles have been assigned to each node. A modifier in Solidity is a piece of reusable code that can be added to a function to alter its behavior. Modifiers frequently enforce limits or conditions on a function, for example, by verifying that the caller of the function has the proper permissions to run it or that a particular need has been satisfied before running the function. In Solidity, modifiers are a specific function for enhancing flexibility and security in your contracts. Following the deployment owner's call to the method "checkContainer," The container's state

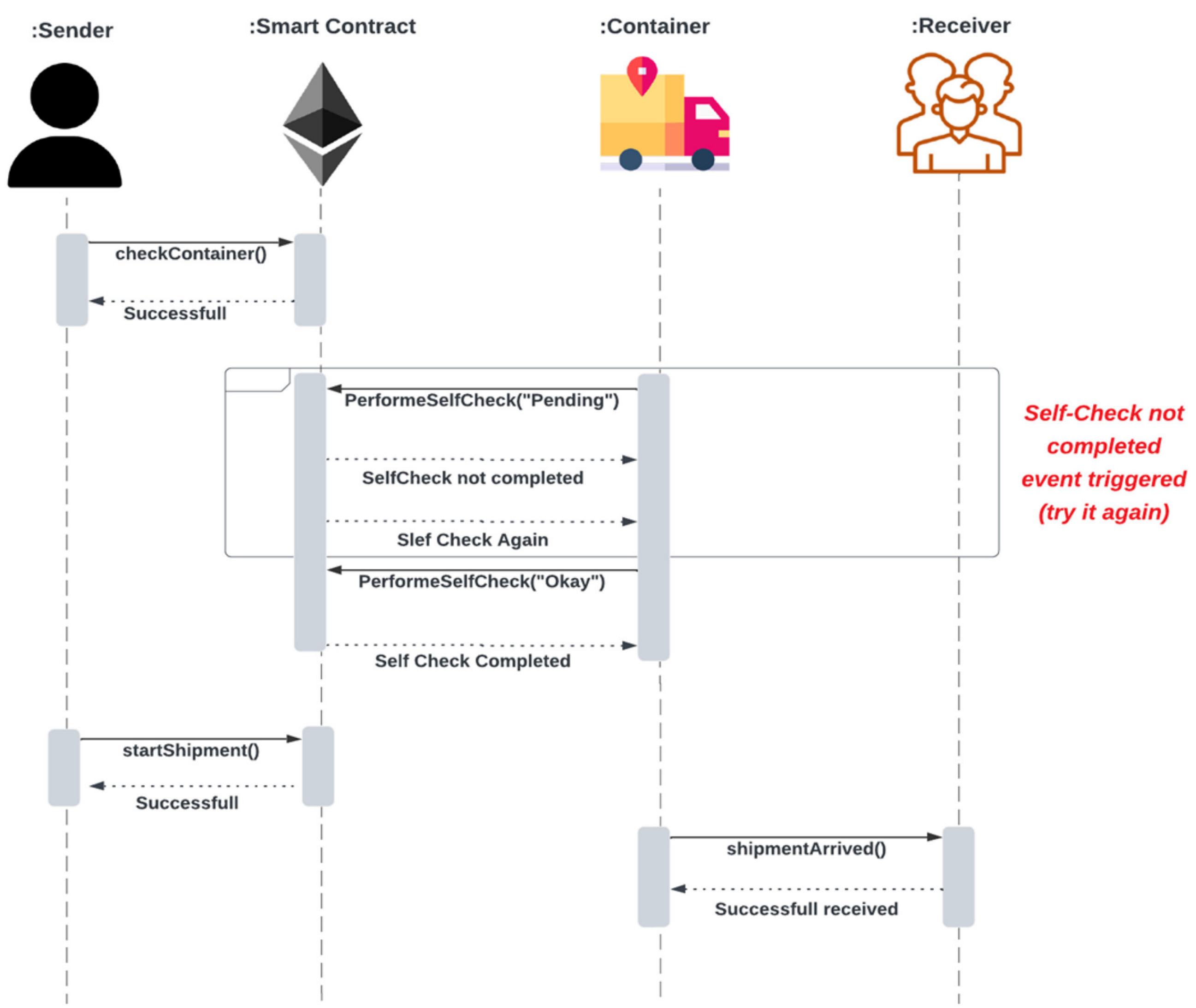

FIGURE 8
Sequence diagram of SFSC.

is changed from "Not Ready" to "Ready State" via this function. Following that, the "performSelfCheck" function will be called. All prerequisites will be verified, such as the sensor being installed, all the objects being loaded, sensor status, and operating conditions. The okay message will be issued if all the requirements are met. If not, the container will once more be examined. Following that, only the receiver can call sendMoney (), as shown in Figure 8, which displays the attributes used in the contract code. Money will flow to the contract owner once the payment procedure is complete, and the invoice will be returned to the sender. The status of the container is updated to "PreparedforDispatch" once the shipment is prepared to begin. The shipment is delivered after the contract owner makes the "StartShippment" call. The receiver will examine the invoice to confirm that the user is valid when the package arrives at its destination. The sender will open the package to inspect the contents after verifying the invoice. The recipient checks the item given to see if it meets all standards, including temperature, food item quality, and timely arrival. The goods will be provided to the purchaser once they are satisfied.

If any violations associated with the enable container are detected after the checkContainer () function has been completed, the shipping container will activate the violationOccurred () function in the smart contract. This function accepts three parameters: a string representing the event's message, an enum representing the type of violation, and the message presenting the violation. This function, when invoked, transforms the status of the package to aborted and refunds the recipient. The sender creates a smart contract to eliminate a third-party role between the source and destination. The Raspberry Pi board then conducts a self-check and invokes the checkContainer () function in the smart contract to continue shipment. If the self-check fails, the container must be checked again, as shown in Figure 8, to ensure that both the sender and the receiver have belief in the source of the container and that it functions without flaws before shipping. Upon shipment delivery, the receiver presents the invoice; after verification, the container will unlock and the shipment can be taken by the customer.

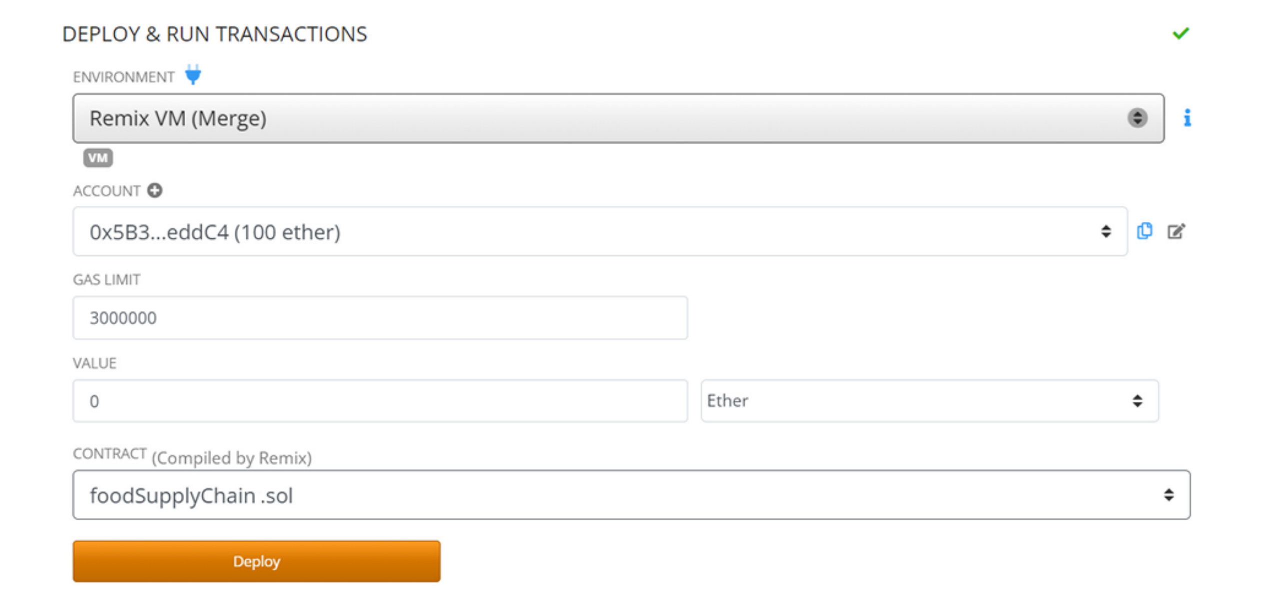

FIGURE 9
Smart contract deployment.

FIGURE 10
Smart contract function of check container.

5 Result and testing

In this section, we will describe the testing strategy for validating the proper operation of three essential features. As shown in Figure 9, we will initially deploy the smart contract on a blockchain network. Once the contract has been effectively implemented, we will confirm that the status of the package has been accurately updated to reflect the flow of the shipment. During testing, we will set the gas limit for sender deposits to 3,000,000 and the transaction value to Ether. After the deployment of the smart contract function checkContainer () will be called, successful execution of this function is shown in Figure 10.

FIGURE 11
Smart contract function for performing self-check (failure).

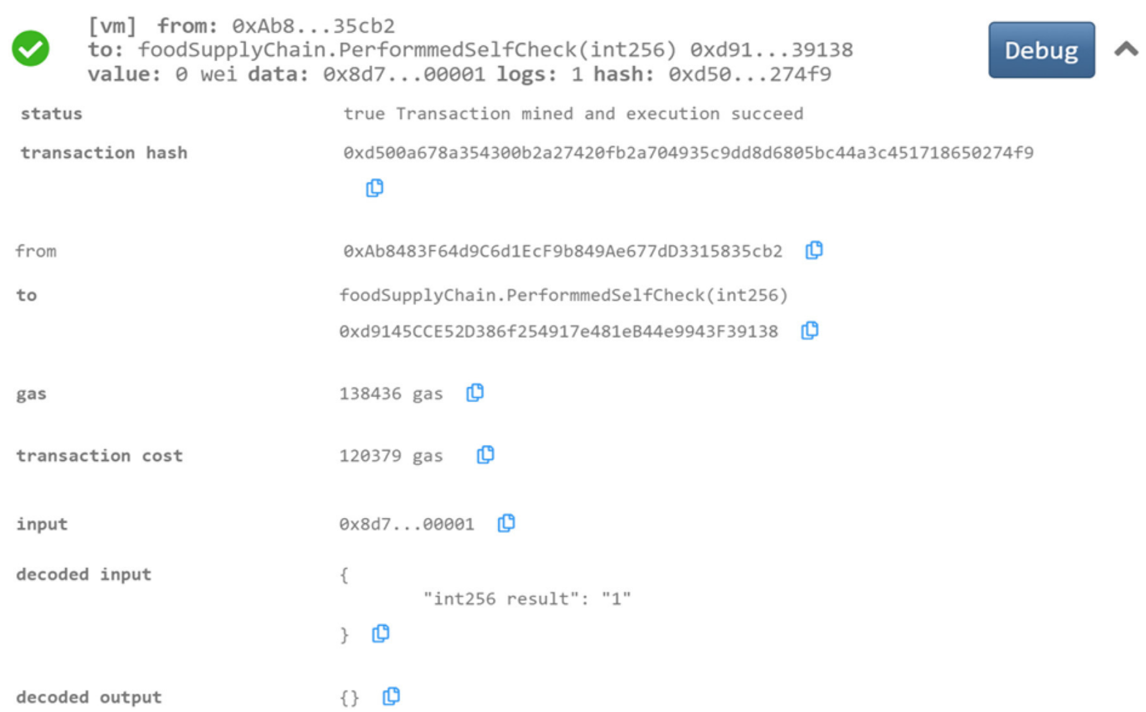

FIGURE 12
Smart contract function for performing self-check (successful).

The second component of our testing strategy involves initiating and logging relevant events. We used three unique Ethereum addresses, each with an initial balance of 100 Ether, to conduct these experiments in Remix. Before executing specific functions, one of the primary focuses of testing was to ensure that the state of the package had been updated accurately. This was

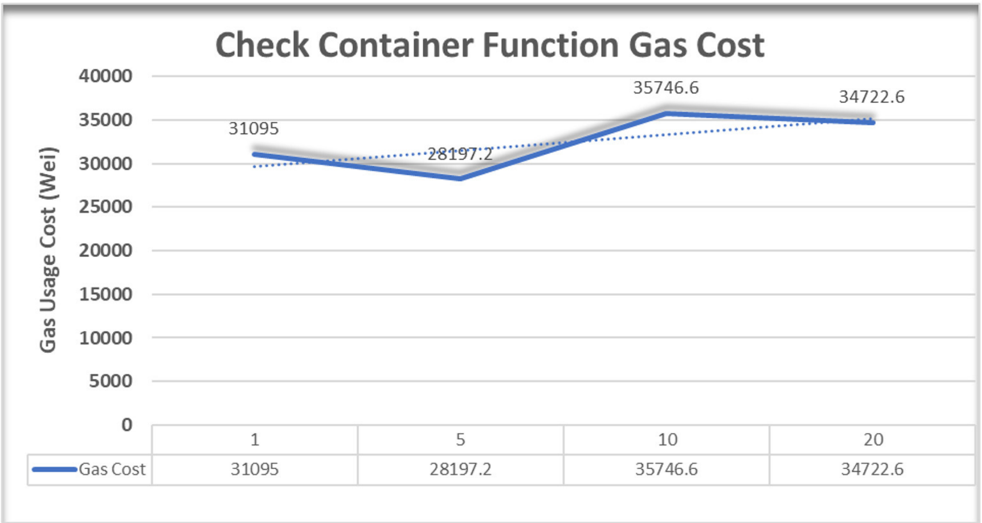

FIGURE 13
Gas cost of smart contract function of check container.

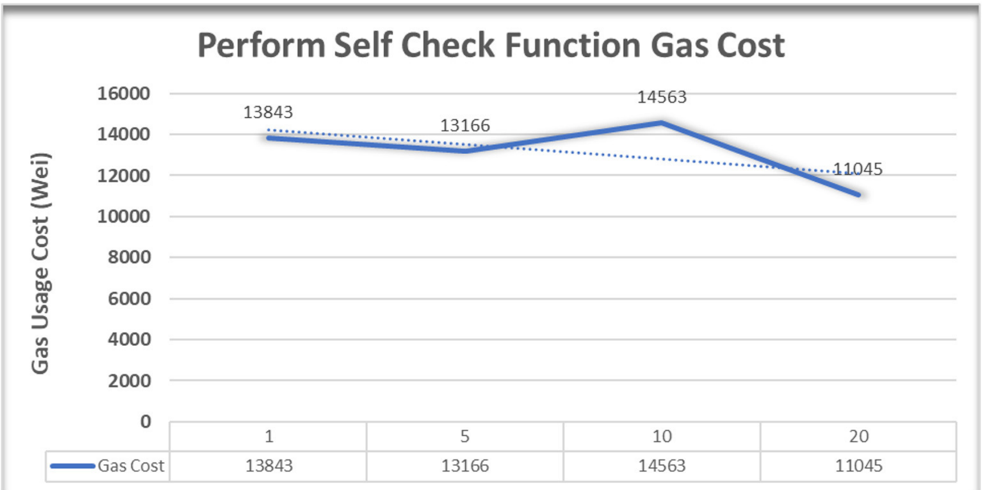

FIGURE 14
Gas cost of smart contract function perform self-check.

essential to ensure the correct execution of flow and order. As shown in Figure 11, an error would be displayed if the proprietor attempted to execute the performSelfCheck () function without the correct state. As shown in Figure 12, if the container address called this function, it would be successfully executed.

A same type of error will occur if a function is called by the incorrect entity, such as instead of calling the recipient, the IoT-enabled shipment container is called. For testing purposes, the price of the shipment is set at 5 Ether. The function sendMoney (address) will always be called by the sender, and Ether will be transferred to the receiver. After effectively executing this function, the log will indicate that "Money deposited and invoice sent to the recipient." This message will only appear in the log if the deposited funds are equal to 5 Ether, and the recipient has also sent an invoice. Once the event is activated, the recipient's address will have 5 less Ether. After the shipment has arrived at its destination, the authorized recipient should verify the correct invoice, examining factors such as temperature, humidity, arrival time, and the freshness of the food. In the event of a violation, the shipment will be rejected, and the user's payment will be refunded via the refundPayment (address) method.

5.1 Function testing

Testing the execution of a smart contract in Ethereum incurs gas costs, which are fees paid to miners for executing the code on the network. To ensure an efficient and cost-effective Solidity code,

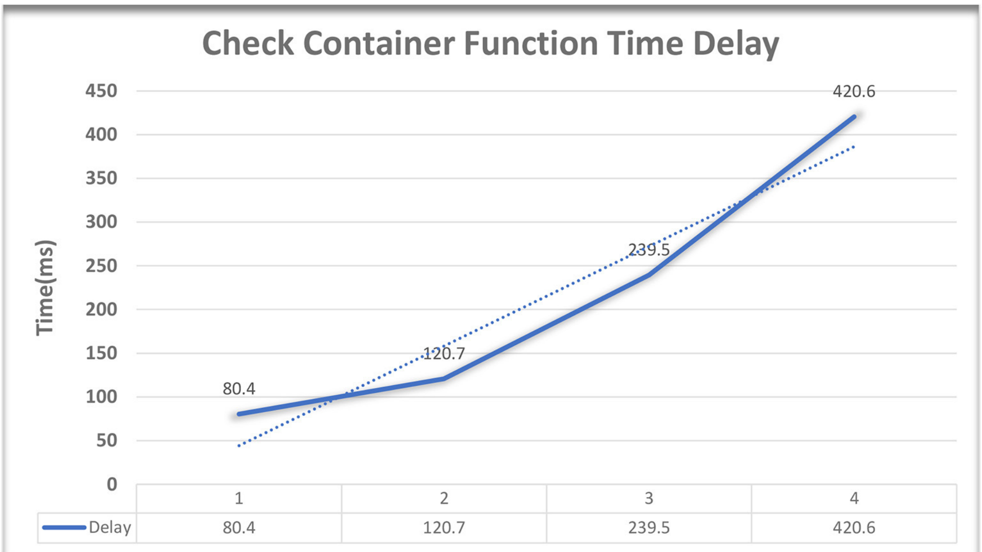

FIGURE 15
Execution time of smart contract function check container.

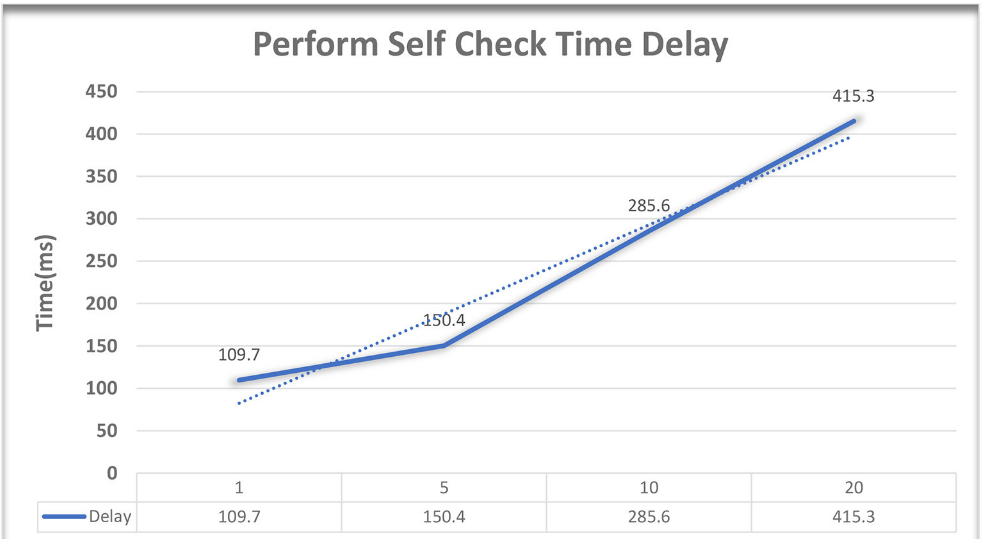

FIGURE 16
Execution time of smart contract function perform self-check.

it is essential to consider the gas costs of various operations. Before deploying the code to the live network, testing it with tools such as Remix and Ganache can help identify potential gas cost issues. The total quantity of gas required to execute a particular transaction is the transaction cost. When evaluating an Ethereum smart contract, it is essential to consider the execution cost of the contract. The cost of execution is the quantity of petroleum required to carry out the contract. Gas is a measurement of the computational effort required to execute the contract, and it is paid in Ether by the contract initiator. To evaluate the cost of a contract's execution, an individual can use the Remix IDE's built-in gas estimator. This tool estimates the quantity of gas required to execute a contract based on the contract's code and input parameters. A contract's execution cost must be evaluated to ensure efficiency and cost-effectiveness. A contract that requires excessive gas to execute may be too costly for users to interact with, resulting in low adoption and utilization.

Remix-Ethereum IDE was used to find out the execution cost, transaction cost, and gas used for testing an Ethereum smart contract. These tools provide information regarding the amount of gas consumed by each function call and the entire transaction cost. A smart contract can be compiled using the Solidity compiler in Remix, then it can be deployed and tested in the Deploy & Run Transactions tab. After each function call, the console will display the amount of petroleum consumed and the transaction cost. Similarly, we write test cases for each smart contract function and execute them via the Remix console. After each test, the console will display the amount of gas consumed and the transaction cost.

In the Ethereum platform and Solidity programming language context, gas cost calculation refers to the amount of computational resources required to execute a specific operation on the Ethereum blockchain. Each operation or transaction on the Ethereum blockchain requires a certain amount of gas to be executed. Gas is a unit of measurement for the computational effort required to complete a transaction. The gas cost of various functions is determined for testing purposes while transporting different shipments. Here, only two smart contract functions of gas cost have been discussed. First, at the initial invocation of the checkContainer () function, the gas cost is "31,095." The average gas cost of a particular operation or transaction can vary depending on factors such as network congestion and the complexity of the operation. Subsequently, after calling this function five times, the average gas cost is "28197.2." After 10 calls, it is 35746.6, and after 20 calls, it is "34722.6," as shown in Figure 13.

The average gas cost of a performeSelfCheck () function has been calculated. After calling this function the first time, the gas cost is "13,843." After calling this function five times, the average gas cost is estimated to be "13,166." After 10 calls, the average gas cost increased to "14,563"; after 20 calls, it decreased to "11,045," as shown in Figure 14.

It is essential to keep in mind that the time execution can change based on the input parameters and the condition of the contract at the time that the execution takes place. Because of this, it is a good idea to test the function with various input parameters to obtain a more accurate estimate of the amount of time it will take to carry out the function.

For the time calculation of functions of a smart contract, two functions have been tested, namely, checkContainer () and performSelfCheck (). Different parameters are used for function testing; for the first time, the checkContainer () function takes "80.4 ms" to execute the function. The average of 5-time execution is "120.7 ms," 10-time execution is "239.5 ms," and 20-time execution of function is "420.6 ms," as shown in Figure 15.

Different parameters are also used for the second function testing; for the first time, the performSelfCheck () function takes "109.7 ms" to execute the function. The average of 5-time execution is "150.4 ms," 10-time execution is "285.6 ms," and 20-time execution of function is "415.3 ms," as shown in Figure 16.

6 Conclusion and future work

In this study, we proposed a blockchain-based FSC solution for the FSC industry. Our proposed methodology is the combination of IoT and blockchain technology, specifically smart contracts, to ensure the delivery of strawberry products of the highest quality to end users. The IoT-enabled container ensures the quality of the strawberry, temperature, humidity, and location of shipment. MQTT cloud-based server continuously fetches all the information from sensors and transfers it to the blockchain network. While our framework emphasizes the SFSC, blockchain-based smart contracts are developed for generic purposes and are also applicable for other fresh good-transporting supply chain networks. Implementation conducted using Remix IDE, the functionality of the smart contract code, concentrating on the cases involving delivery trustworthiness, breach detection throughout transportation. Furthermore, the proposed system can be modified by deploying our smart contracts on the Hyperledger platform and developing Decentralized Applications (dApps) that integrate real-world entities in a supply chain.

Data availability statement

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

Author contributions

MG: Data curation, Formal analysis, Methodology, Resources, Validation, Visualization, Writing—original draft. MK: Conceptualization, Data curation, Formal analysis, Investigation, Writing—original draft. AH: Formal analysis, Investigation, Methodology, Validation, Writing—original draft. HA: Investigation, Methodology, Software, Visualization, Writing—original draft. MS: Conceptualization, Methodology, Supervision, Writing—review & editing.

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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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Quality and bioactive compound accumulation in two holy basil cultivars as affected by microwave-assisted hot air drying at an industrial scale

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Holy basil (*Ocimum Tenuiflorum* L.) contains several bioactive compounds useful to the pharmaceutical and food industries. Microwave drying (MD) is a powerful technique for rapid drying of food or plant materials while preserving bioactive compounds during the process. However, little is known about the optimal combination of MD power with hot air drying (HAD) that can preserve the quality and yet only consume reasonable energy when drying holy basils. For that purpose, the effects of drying methods using MD combined with HAD at 45°C were examined to prevent losses in quality, antioxidant activities, and volatile flavor compounds in two holy basil cultivars (green and red cultivars). Holy basil leaves were dried at different MD powers of 200, 400, and 600 W combined with HAD and compared with a traditional tray drying (TD) at 45°C. Drying using MD at 600 W with HAD displayed significantly high levels of color retention, chlorophyll, and carotenoid content in both cultivars. The green cultivar showed a greater accumulation of total phenolic compounds (TPC), terpenoids, and DPPH free radical scavenging at 400 W with HAD. However, the red cultivar had the highest TPC, flavonoid, and terpenoid content at 600 W with HAD. The accumulation of major volatile organic compounds (VOCs) was also affected, and treatment at 600 W exhibited the highest methyl eugenol and β-caryophyllene content in both cultivars. The use of the highest power of MD (600 W) with HAD for leaf drying reduced the effective drying time and energy consumption among both cultivars. Taking into consideration the dried quality of antioxidant accumulation and energy consumed for drying, we recommend using MD at 400 or 600 W with HAD for the green cultivar and 600 W for the red.

KEYWORDS
Ocimum Tenuiflorum L., secondary metabolite, plant factory, bioactive compounds, drying methods, tray drying

Introduction

The genus *Ocimum* is a member of the family Lamiaceae, comprising approximately 68 species indigenous to tropical regions of Asia, Africa, and Central and South America (Singh and Chaudhuri, 2018). Holy basil, *Ocimum sanctum* Linn. synonym *Ocimum tenuiflorum* L., is the most important species of the genera due to its medicinal, perfumery, religious, ceremonial, cuisine, and essential oil uses (Nandkarni, 1982). This aromatic shrub is commonly identified as two cultivars, namely, the green holy basil with green leaves and red holy basil with purple leaves (Darrah, 1974; Vani et al., 2009). Holy basil is reported to exhibit antidiabetic, wound-healing, antioxidant, radiation protective, immunomodulatory, antifertility, anti-inflammatory, antimicrobial, antistress, and anticancer activities (Godhwani et al., 1988; Kelm et al., 2000; Vats et al., 2004; Yanpallewar et al., 2004; Mukherjee et al., 2005; Singh et al., 2005; Gupta et al., 2006; Salles Trevisan et al., 2006). The aroma compounds of holy basil essential oil (methyl eugenol chemotype, 56.18%) have been identified by solid phase microextraction (SPME)/GC–MS/flame ionization detection (FID) and olfactoric evaluations. The spicy-green-notes of essential oil are due to methyl eugenol, β-caryophyllene oxide, and germacrene D, while spicy-peppery-notes correspond to germacrene D (Jirovetz et al., 2003). Moreover, the major pharmacological activities of holy basil essential oil, such as mosquitocidal, antimicrobial, and anthelmintic, were found to be mediated by its marker constituent, eugenol (Kelm and Nair, 1998; Asha et al., 2001; Kumar et al., 2010).

Fresh leaves of plants are highly perishable and tend to lose quality immediately after harvest and thus require preservation against deterioration and spoilage. Water content in fresh Lamiaceae herbs is approximately 75–80% wet basis; however, to preserve its quality, water levels need to be reduced to less than 15% wet basis for preservation (Dixon and Paiva, 1995). Drying is one of the oldest and the most commonly used preservation techniques. Drying the herbs inhibits microbial growth and forestalls certain biochemical changes. Yet, it can cause alterations that affect herb quality, such as changes in structure and alterations in aroma caused by losses in volatiles or the formation of new volatiles as a result of oxidation or esterification reactions (Díaz-Maroto et al., 2002; OrphAnides et al., 2013). Certain compounds have been observed to increase in different herbs after drying—for example, eugenol in bay leaf (Díaz-Maroto et al., 2002; OrphAnides et al., 2013), thymol in thyme (Venskutonis, 1997), and some sesquiterpenes in different herbs (Baritaux et al., 1992; Yousif et al., 1999). Most studies have reported changes in the color and volatile compounds of aromatic herbs after drying (Díaz-Maroto et al., 2002; Di Cesare et al., 2003). The quality of the dried product is mostly influenced by drying conditions as well as the method adopted for drying. Currently, most dehydration techniques that are used to preserve fruits are fluidized bed drying, solar drying, hot air drying, microwave drying, osmotic dehydration, foam mat, spray drying, and freeze-drying (Barrozo et al., 2014).

Tray drying is the conventional method of drying that is widely used on a commercial scale because of its simplicity and low-cost equipment. However, tray drying involves a longer drying time due to the requirements of convective hot air. In addition to longer drying durations, the products are also exposed to undesirable effects such as reductions in bioactive compounds, shrinkage, slow rehydration, and loss of color (Dev et al., 2011; Workneh and Oke, 2012; Horuz and Maskan, 2015; Hihat et al., 2017; Kaveh et al., 2021; Zia and Alibas, 2021). Hence, the use of microwaves has emerged as an interesting alternative technique. In contrast to convective drying, microwaves can generate large amounts of heat inside rather than externally. Heat is generated by water molecules interacting with electromagnetic waves directly, leading to water vapor pressure differences between the interior and surface areas, leading to rapid external migration of moisture.

Microwave-assisted hot air has been widely used for agricultural drying with a positive effect on quality, antioxidant activity, and bioactive compounds (Arslan and Özcan, 2010; Dev et al., 2011; Workneh and Oke, 2012; Chaikham et al., 2013; Ozcan-Sinir et al., 2018; Poogungploy et al., 2018). Sensory evaluation of persimmon chips processed by microwave-assisted hot air microwave and freeze techniques showed higher sensory scores and nutritional values than traditional hot air drying (Jia et al., 2019). This indicated that dried samples using microwave techniques were positively affected by food products. In recent times, the application of microwave hot air rolling drying (MHRD) has developed to incorporate the advantages of microwave drying and hot air drying. The MHRD technique could improve the cooking efficiency of kidney beans, with a reduction of 2.5 times drying time at the same temperature when compared to without microwaves (Li et al., 2022). It has also been applied in ginger drying to determine the appropriate microwave power and drying temperature for the final product (Zeng et al., 2023). Similarly, microwave drying was applied for dewatered sludge, and it was found that the application of microwaves reduced the drying time by at least 37.5% compared to using hot air (Wulyapash et al., 2022). In addition to quality prospectus, concerns that producers are preoccupied with are energy usage that influences drying costs. These concerns may be reduced by microwave-assisted hot air drying (Poogungploy et al., 2018; Kaveh et al., 2021; Yue et al., 2021).

This study, therefore, aimed to evaluate the effect of alternative microwave drying processes under different conditions while protecting and maintaining dried leaf quality and bioactive compound accumulation in the red and green holy basil cultivars. We investigated the energy consumption at an industrial scale for food production and cosmetics industry use.

Materials and methods

Plant material

The fresh stems and leaves of two commercial holy basil cultivars (*Ocimum tenuiflorum* L.; green and red holy basil) were collected at the harvesting stage (49 days after transplantation) from a plant factory using artificial light (PFAL) at the National Center for Genetic Engineering and Technology Development Agency (BIOTEC), Thailand Science Park, Klong Lung, Pathum Thani, Thailand. Holy basil plants were grown under controlled environmental conditions with artificial light at 200 $\mu mol m^{-2} s^{-1}$ of light intensity, 25 ± 1°C air temperature, 75 ± 3% relative humidity, and 1,000 ± 150 $\mu mol\ mol^{-1}$ CO_2 concentration (Thongtip et al., 2022; Chutimanukul et al., 2022b).

Drying methods and energy efficiency

The leaves of green and red holy basil were dried under four different conditions, each with four replications. The drying methods comprised commercial tray drying (TD) with hot air at 45°C and previously unevaluated regimes of microwave drying (MD) assisted hot air drying at 45°C under three different power strengths of 200, 400, and 600 W. The TD with hot air for industrial scale is presented in Figure 1A. We used 12 drying trays with width, length, and height of 53 × 72 × 3 cm. MD was designed and constructed by staff from the Faculty of Engineering, Mahasarakham University, Thailand. The apparatus for microwave-assisted hot air drying at the pilot scale is shown in Figure 1B. Four magnetrons at 1000 W were installed, generating microwaves at a frequency of 2,455 MHz. Under MD, the air was heated by a 9 kW electric heater and circulated by a 1.5 kW centrifugal fan with an electric motor. The drying chamber was 80 cm each in length, width, and height and featured eight drying trays with a diameter of 60 cm. Plant material samples for each run were 500 g placed on rotated trays, and fresh holy basil leaves were laid out in a single layer for drying with a thickness of less than 2 mm. Samples were dried until water activity (a_w) was lower than 0.6 (i.e., the recommended safe level for storage). After drying, the weight of each sample was recorded to calculate water evaporation.

Moisture content and a_w determination

Fresh leaves were chopped into small pieces before determining moisture content. Approximately 5 g samples were placed in an aluminum can and put in the oven at 103°C for 24 h. Moisture content was calculated from the water evaporated divided by the fresh weight (AOAC, 1995). The dried samples were measured for a_w using an AquaLab water activity meter (Aqua-Link 3.0, Pullman, WA). It was calibrated with distilled water to obtain a_w in the range of 1.000 ± 0.003. Five dried leaves were used for each measurement.

Specific energy consumption

SEC is the total energy consumed during drying divided by the mass of water evaporation. Tray drying energy consumption was evaluated from the blower and heater apparatus. The energy consumption of the microwave-assisted hot air drying was determined from four components, namely, microwave, blower, motor, and heater apparatus. A clamp multimeter was used to measure the voltages and electrical currents of all apparatus. The electrical energy consumption of the magnetron and heater was calculated by

$$Emicrowave\ or\ Eheater = \left(\mathrm{V} \times \mathrm{I} \times t_1\right) / 1000 \quad (1)$$

The electrical energy consumption of the blower and motor was calculated by

$$Eblower\ or\ Emotor = \left(\mathrm{V} \times \mathrm{I} \times t_2\right) / 1000 \quad (2)$$

where E is the energy consumption of each component (kWh), V is voltage (V), I is electrical current (A), t_1 is time (h) when the device is turned on during drying, and t_2 is drying time (h) throughout the experiment.

The SEC of the microwave-assisted hot air dryer was reported in MJ/g of water evaporation using the following expression:

$$SEC = \frac{3.6\left(E_{microwave} + E_{heater} + E_{blower} + E_{motor}\right)}{m_i - m_f} \quad (3)$$

The SEC of the tray dryer was reported in MJ/g of water evaporation using the following expression:

$$SEC = \frac{3.6\left(E_{heater} + E_{blower}\right)}{m_i - m_f} \quad (4)$$

where 3.6 is the electric energy conversion factor from kilowatt hour to megajoule, m_i is initial mass and m_f is the final mass (g).

Color measurements

Leaf samples of both green and red cultivars were ground to a fine powder before measuring color with a Hunter Lab Colorimeter (type Color Flex, United States). Color coordinate values, L^*, a^*, b^*, for each sample were recorded. L^* represented lightness, varying from 0 (dark) to 100 (light). Positive a^* and b^* values indicated redness and yellowness, while negative a^* and b^* values indicated greenness and blueness. Chroma (C) and hue angle (h) were calculated using the following equations (Pathare et al., 2013):

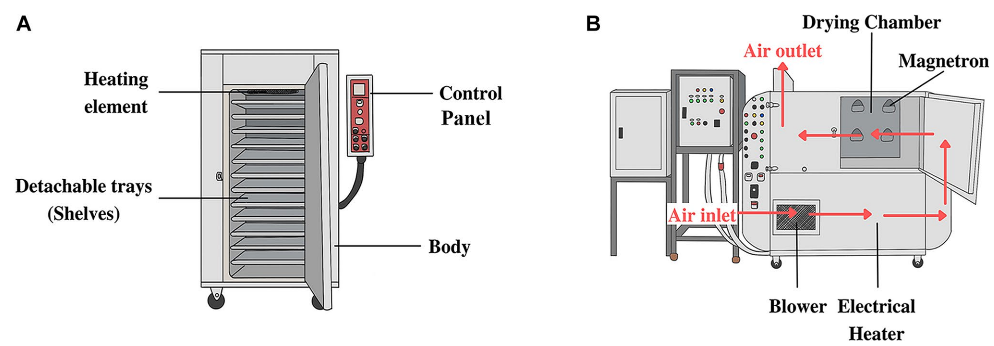

FIGURE 1
The pilot scale of the drying process of commercial tray drying with hot air **(A)** and microwave-assisted hot air dryer with the airflow direction **(B)**.

$$C = \sqrt{a^{*2} + b^{*2}} \quad (5)$$

$$h = \arctan \frac{b^*}{a^*} \quad (6)$$

Pigments accumulation

All pigment contents such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the holy basil were examined according to Wellburn (1994). The dry samples were ground to a fine powder in a mortar, from which 100 g was extracted with 1 mL of absolute methanol solvent. The mixture was then incubated at 15°C in darkness for 72 h and then centrifuged at 8,000 rpm, 15°C for 5 min. After centrifugation, the supernatant solution was separated completely. The absorbance of the extracted solution was recorded at wavelengths of 470, 646.8, and 663.2 nm using a spectrophotometer (Agilent 8,453 UV–vis Spectroscopy System, Country). The concentration of the pigments was calculated using the following formulae (Wellburn, 1994):

$$\text{Chlorophyll a}\left(\text{Chl a}\right) = 12.25\ \text{A}663.2 - 2.79\ \text{A}646.8 \quad (7)$$

$$\text{Chlorophyll b}\left(\text{Chl b}\right) = 21.5\ \text{A}646.8 - 5.1\ \text{A}663.2 \quad (8)$$

$$\text{Carotenoids} = \left(1{,}000\ \text{A}470 - 1.82\ \text{Chl a} - 85.02\ \text{Chl b}\right) / 198 \quad (9)$$

$$\text{Total Chlorophyll}\left(\text{TChl}\right) = \text{Chl a} + \text{Chl b} \quad (10)$$

Pigment accumulation was presented as ug per gram dry weight (DW) of the sample.

Antioxidant activity evaluation

Sample extraction

Holy basil samples, after harvesting and drying by different methods, were ground to a fine powder and extracted following a modified method (Chutimanukul et al., 2022a) for analyzing the total content of phenolic compounds (TPC), total flavonoid compounds (TFC), and the scavenging activity of DPPH radicals. Ten microgram of the fine powder sample was thoroughly mixed with 5 mL absolute methanol containing 1% HCL, which was used as a solvent. After incubation at room temperature for 3 h, the mixture solution was centrifuged for 5 min at 12,000 rpm. The supernatant was transferred to another microtube and used for the determination of TPC, TFC, and DPPH radical scavenging activity.

Total phenolic content

The total phenolic content (TPC) was assayed following modified Folin-Ciocaltea colorimetric methods (Chutimanukul et al., 2022b). Two hundred microliter of extracted solution was mixed with 200 µL of 1 N Folin–Ciocalteu reagent and incubated at room temperature for 15 min. Then, the mixture was added to 600 µL of 7.5% sodium carbonate (Na_2CO_3) and incubated for 1 h before detecting the absorbance at 730 nm by spectrophotometer (MultiskanSky, Thermo Scientific). Gallic acid was used as a standard solution, and the results were presented as milligrams of gallic acid equivalent per gram dry weight of the sample (mg of GAE / g DW).

Total flavonoids content

The quantification of total flavonoid content in holy basil was evaluated based on the method of (Chutimanukul et al., 2022b) with minor modification of the colorimeter method. Three hundred and fifty microliter of the extracted solution was added to 75 µL of 5% sodium nitrite ($NaNO_2$) and completely mixed using a vortex. The mixture was incubated for 5 min before adding 75 µL of 10% aluminum chloride ($AlCl_3{\cdot}6H_2O$) and kept at room temperature for 5 min. Lastly, 1 M sodium hydroxide (NaOH) was pipetted to the mixture solution and left to stand for 15 min. The absorbance of the homogenate solution was determined at 515 nm by a spectroradiometer. The content of total flavonoids was calculated using rutin as the standard solution and showed as milligrams of rutin equivalent per gram dry weight of holy basil (mg of rutin/g DW).

Total terpenoids content

To investigate the content of total terpenoids in the holy basil after drying, 100 mg of fine powder of the dried sample from several drying treatments was extracted by mixing with 1 mL absolute methanol and incubating in the dark for 48 h. After incubation, the mixture was centrifuged at 6,500 rpm for 15 min. The determination of total terpenoids of the extracted solution was performed using the procedures of Ghorai et al. (2012) and Chutimanukul et al. (2022a). Absorbance was measured at a wavelength of 540 nm using a spectrophotometer with 99.9% methanol as a blank control. Linalool was used as the standard solution, and the content of total terpenoids was exhibited as milligrams of linalool per gram dry weight (mg of linalool /g DW).

DPPH radical scavenging activity

Antioxidant scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was detected by following the method of Chutimanukul et al. (2022b). Hundred microliter of extracted solution was completely mixed with 900 µL of 0.1 mM DPPH and kept in the dark at 25°C for 30 min. The absorbance of the mixture was determined at 515 nm using a spectroradiometer. Trolox was used as a reference antioxidant. The percentage of DPPH scavenging as an antioxidant activity was calculated by the equation (Yen and Duh, 1994): Inhibition% = $(A_{control} - A_{515})/(A_{control}) \times 100$. Where $A_{control}$ and A_{515} were the absorbance of 0.1 mM DPPH without sample and absorbance of sample at wavelength 515 nm, respectively.

Volatile compound quantification

The analysis of volatile organic compound (VOCs) content was obtained by gas chromatography with mass spectrometry (Agilent; 7890B) combined with a quadruple time-of-flight mass spectrometer (GC/Q-TOF, Agilent, 7250) and PAL autosampler system (CTC Analytics AG, Switzerland). Extraction of the sample and analysis were carried out according to the method described by Chutimanukul et al. (2022a). Hundred milligram of the dried leaf powder was placed in 1 mL of absolute methanol with 10 μL of 2000 ppm gamma-hexalactone, and then uniformly mixed for 1 min. The samples were sonicated by ultrasonic bath for 30 min. To eliminate the insoluble material, the prepared extracts were centrifuged at 10,000 rpm for 5 min. The supernatant was collected and stored in a vial at −20°C for further analysis.

For the analysis of VOCs of dried leaves of holy bail in each MD treatment, 1 μL of the extract was injected into a multimode inlet (MMI). The inlet temperature was controlled at 250°C, and the samples were carried at a constant flow rate of 1.0 mL/min by high-purity helium (>99.999%). Chromatographic separation was conducted with a DB-FFAP column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, United States). GC operational conditions were as follows: the temperature program was initiated at 60°C for 1 min, increased to 250°C by 10°C/min for 3 min; transfer line, 250°C; ion source (EI) temperature, 240°C; quadruple temperature, 150°C; EI energy 70 eV; and scanning mode from 20 to 350 m/z. Four target compounds, including eugenol, methyl eugenol, β-Caryophyllene, and Linalool, were investigated. The calibration curves were constructed with the relative internal response ratio as a function of the analyte concentration of the mixtures of the four target standard compounds with a range of 0.04–100 ppm.

For data processing, the target compounds, including eugenol, methyl eugenol, and α-Humulene, were determined by comparing the calibration curves against relative internal response ratios. Concentrations of target analytes were reported as μg per gram of DW.

Experimental plan and data analysis

The experiments were designed according to a completely randomized design (CRD) with four replications. Statistical data were analyzed by IBM SPSS (IBM Corporation; Armonk, NY, United States). One-way analysis of variance (ANOVA) was used for sample comparisons in each parameter. Mean separations were analyzed with Duncan's Multiple Range Test (DMRT) at a 95% significance ($p < 0.05$). The data were presented as means ± S.E (standard error).

Results

Energy consumption

To investigate the energy consumption and specific energy consumption of the four different drying treatments on the two cultivars of holy basil, the initial moisture content, final moisture content, and water activity (a_w) of leaves after drying were calculated. Table 1 indicates the effects of the four drying methods: microwave drying (MD) under three different powers of 200, 400, and 600 W combined with hot air drying (HAD) at 4°C and the tray drying (TD) method using 45°C of hot air affected drying time. The total drying times for TD were substantially longer in comparison to microwave oven drying of both cultivars. Among different powers for MD treatments, the highest MD power caused the lowest average drying time. For a load of 500 g fresh leaves at 200, 400, and 600 W of MD with HAD, green holy basil leaves were dried after 6, 3.5, and 3 h, respectively, while the red holy basil leaves took 5, 4, and 2.5 h, respectively. Moreover, across drying methods, the drying process caused reductions in moisture contents of fresh leaves, from between 87.5 and 88.6% at harvest to 8.0–9.9% in 2.5–7 h of drying. After drying, the observed a_w value ranged from 0.30 to 0.45. The appearance of two holy basil cultivar leaves after drying treatments is shown in Figures 2, 3.

Energy efficiency via evaluation of energy consumption and specific energy consumption (SEC) was assessed. Blower (E_{blower}), motor (E_{motor}), heater (E_{heater}), microwave ($E_{mocrowave}$), total energy (E_{total}), evaporated water, and SEC were considered. The details are presented in Table 2. After drying two cultivars of holy basil, we found that the MD treatments had the lowest E_{blower}, E_{heater}, $E_{total,}$ and SEC as the power of the microwave increased. On the other hand, TD had the highest value. Among different drying treatments, there were no differences in the values of evaporated water. Compared to the values obtained through the TD treatment, the SEC value at 600 W of MD was 3.5 times in green holy basil and 4 times in red holy basil.

Qualitative properties

The color change of green and red holy basil dried leaf powder is shown in Table 3. Overall, lighter-colored leaf samples were dried with the highest MD power. For dried leaves of both green and red cultivars, the parameters of L^* (Lightness) value of MD at 600 W were the highest when compared with other methods. When comparing the a^* values of all dried leaves to those of fresh leaves, the lowest a^* (redness: green to red) values were observed under MD at 600 W, while TD showed the highest value. Moreover, b^* (yellowness) of both cultivars showed the lowest value under 600 W. C (chroma value: color saturation) and h (hue angle: color angle) increased with an increase in the power of MD, with leaf samples becoming clearer after drying. For MD at 200 and 400 W, dried leaf samples of each cultivar appeared lighter and greener than for TD treatment.

Pigment accumulation

The effect of different drying techniques on pigment content was investigated (see Figure 4 for details). Leaves of green and red holy basils were significantly influenced by drying treatment. A significant accumulation of total chlorophyll (TChl), chlorophyll a (Chl a), and chlorophyll b (Chl b) was identified in both cultivars. It was the highest at 600 W MD (Figures 4A–C). Additionally, MD at 600 W resulted in increased carotenoid content in dried leaves of green holy basil, while carotenoid content was lowest when leaves were dried under TD. However, the red cultivar did not show significant differences across drying treatments (Figure 4D).

Bioactive compounds and antioxidant capacity

After drying, the content of total phenolic compounds (TPC), total flavonoid content (TFC), and total terpenoids were found to be affected by the different drying treatments (Figure 5). The TPC content of both green and red holy basil leaves showed significant differences. Green holy basil leaves showed the highest TPC levels at 400 W of MD, while red holy basil leaves had the highest TPC content

TABLE 1 Drying time, initial moisture content, final moisture content, and water activity (a_w) of green and red holy basil leaves after drying by tray drying (TD, 45°C) and various microwave powers combined with hot air drying at 45°C (HAD) (200 W + 45°C, 400 W + 45°C and 600 W + 45°C).

Holy basil cultivar	Drying treatment	Drying time (h)	Initial moisture content (% w.b.)	Final moisture content (% w.b.)	a_w
Green	45°C	7	88.12 ± 0.45	9.66 ± 0.04	0.40 ± 0.04
	200 W + 45°C	6	88.26 ± 0.70	9.02 ± 0.03	0.39 ± 0.02
	400 W + 45°C	3.5	87.93 ± 0.06	9.25 ± 0.29	0.36 ± 0.04
	600 W + 45°C	3	87.89 ± 1.39	8.68 ± 0.97	0.33 ± 0.03
Red	45°C	6	88.12 ± 0.91	9.06 ± 0.37	0.37 ± 0.02
	200 W + 45°C	5	88.55 ± 0.09	8.42 ± 0.41	0.33 ± 0.01
	400 W + 45°C	4	88.25 ± 0.40	8.82 ± 0.25	0.35 ± 0.03
	600 W + 45°C	2.5	88.22 ± 0.37	9.83 ± 0.04	0.43 ± 0.02

Values are represented as mean ± SE (n = 4).

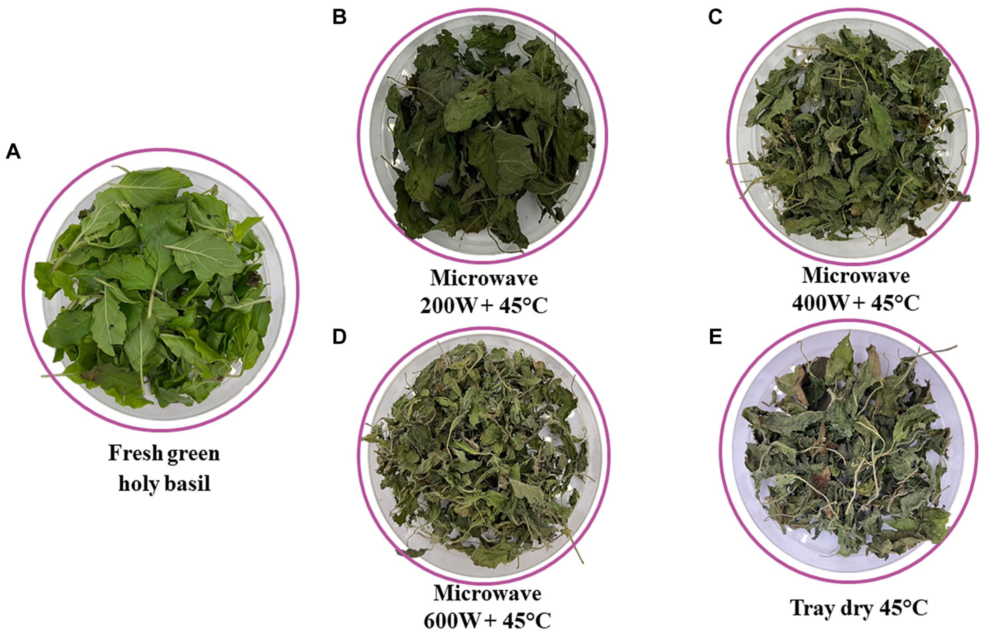

FIGURE 2
Quality retention of green holy basil leaves after drying by various microwave power combinations with hot air at 45°C (HAD), fresh holy basil **(A)**, 200 W + 45°C **(B)**, 400 W + 45°C **(C)**, 600 W + 45°C **(D)** and tray drying **(E)**.

at 600 W (Figure 5A). Across different drying treatments, there were no significant differences for TFC in green holy basil. However, different drying treatments affected TFC accumulation in red basil, with 600 W MD showing the highest accumulation (Figure 5B). Different drying treatments significantly influenced the total terpenoid content in both green and red holy basil leaves (Figure 5C). MD at 400 and 600 W revealed the highest values for green holy basil leaves, and the levels were highest at 600 W in red holy basil. The total terpenoid content was lowest in TD treatments for red holy basil. DPPH analysis among drying treatments showed that antioxidant activity, i.e., the scavenging rates of DPPH, in green holy basil leaves had the highest value at MD 200 W. However, it was not significantly different than MD 400 W. Further, MD at 600 W recorded the lowest reading. Drying treatment did not significantly affect the antioxidant capacity among red holy basil leaves (Figure 5D).

Volatile content

Volatile organic compounds (VOCs) of the two holy basil cultivars were analyzed using gas chromatography coupled with mass spectrometry (GC/Q-TOF). Results were obtained for the following VOCs: eugenol, methyl eugenol, Linalool, and β-caryophyllene (Figure 6). The highest eugenol levels for green holy basil were observed in TD treatments, followed by 200, 400, and 600 W MD, respectively (Figure 6A). However, red holy basil leaves did not show significant differences among drying treatments. In contrast, a significantly greater amount of methyl eugenol was present at 400 and 600 W MD in green holy basil leaves, but it was not significantly affected in red holy basil leaves (Figure 6B). Linalool alone was significantly different in the green holy basil leaves (Figure 6C). Regarding β-caryophyllene, TD and 200 W MD recorded the highest

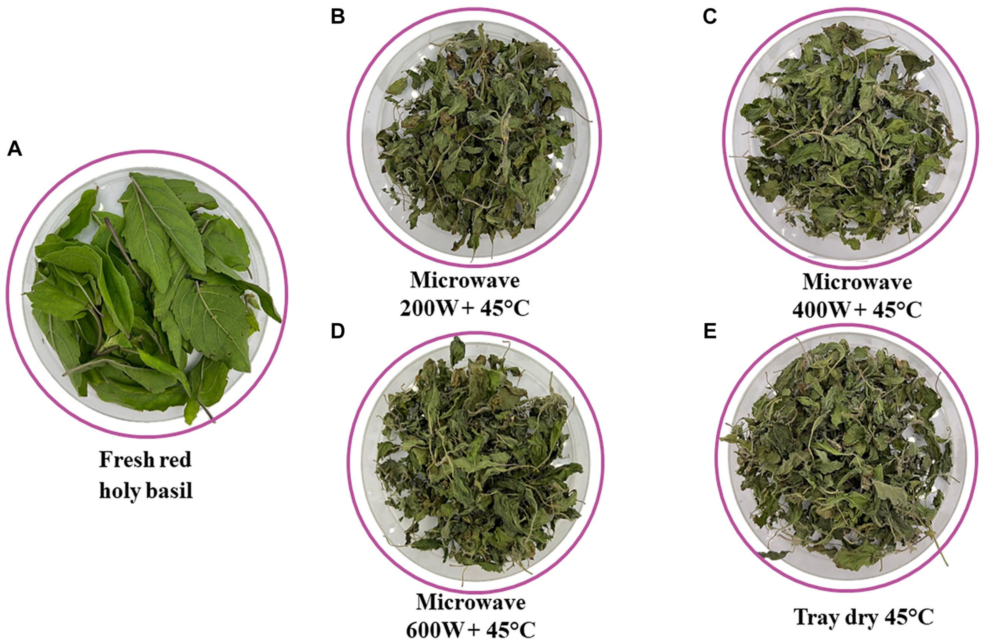

FIGURE 3
Quality retention of red holy basil leaves after drying by various microwave power combinations with hot air at 45°C (HAD), fresh holy basil **(A)**, 200 W + 45°C **(B)**, 400 W + 45°C **(C)**, 600 W + 45°C **(D)** and tray drying **(E)**.

TABLE 2 Energy consumption values of green and red holy basil leaf samples with tray drying (TD, 45°C) and various microwave powers combined with hot air drying at 45°C (HAD) (200 W + 45°C, 400 W + 45°C, and 600 W + 45°C).

Holy basil cultivar	Drying treatment	Drying time (h)	E_{blower} (kWh)	E_{motor} (kWh)	E_{heater} (kWh)	$E_{microwave}$ (kWh)	E_{total} (kWh)	Evaporated water (g)	SEC (MJ/g)
Green	45°C	7	4.95	–	38.78	–	43.73	78.46	2.01
	200 W + 45°C	6	1.12	0.70	15.94	7.20	24.96	79.24	0.86
	400 W + 45°C	4	0.75	0.47	11.95	6.40	19.57	78.68	0.68
	600 W + 45°C	3.5	0.65	0.41	9.30	7.35	17.71	79.21	0.57
Red	45°C	6	4.24	–	33.24	–	36.48	79.06	1.71
	200 W + 45°C	5	0.93	0.58	13.28	5.00	19.79	80.13	0.71
	400 W + 45°C	4	0.75	0.47	11.95	6.40	19.57	79.43	0.67
	600 W + 45°C	2.5	0.47	0.30	6.64	3.75	11.6	78.39	0.41

values, and MD 400 and 600 W showed the lowest β-caryophyllene values, which were significantly different for both holy basil varieties (Figure 6D). MD 600 W displayed the highest content in green holy basil leaves but was not significantly different from the TD treatment. β-caryophyllene content was highest in MD 400 and 600 W.

Discussion

Our study indicates that variations in microwave drying treatments have significant impacts on the energy consumption of green and red holy basil leaves at an industrial scale. According to the industrial standards for dried herbal materials, the final moisture content is usually below 12% w.b., depending on the type of plant tissue (Preparations WECoSfP and World Health Organization, 1996). Such moisture content typically enhances shelf life during storage without affecting the quality of the plant products by reducing their susceptibility to post-harvest pathogens (Poós and Varju, 2017). Several studies have shown that a moisture content range of 7–12% is ideal for raw herbal materials in medicine preparations (Tarhan et al., 2011; Jayaraman and Gupta, 2020; Shalaby et al., 2020; Wiset et al., 2021). Consequently, the drying method is an important factor that has a considerable impact on the quality of resulting herbal materials. Our holy basil leaf samples from the two cultivars under plant factory cultivation showed 8.0–9.9% final moisture content after the various drying treatments (Table 1). We dried leaf samples until a_w was lower than 0.6, which is safe for storage and prevents microbial growth (Rahman and Labuza, 2007).

Overall, the increased power of MD was associated with reduced drying times of the herbal products. Microwaves increase moisture evaporation rates from plant tissues by the rapid activation of energy absorption of water molecules. This leads to the generation of a

TABLE 3 The color parameters (L^*, a^*, b^*, C, and h) of green and red holy basil leaves after drying using tray drying (TD, 45°C) and various microwave powers combined with hot air drying at 45°C (HAD) (200 W + 45°C, 400 W + 45°C, and 600 W + 45°C).

Holy basil cultivar	Drying treatment	L^*	a^*	b^*	C	h
Green	45°C	40.30 ± 0.24[a]	−4.77 ± 0.04[a]	20.95 ± 0.29[c]	21.39 ± 0.69[c]	101.62 ± 0.14[b]
	200 W + 45°C	39.79 ± 0.62[b]	−5.56 ± 0.02[b]	22.12 ± 0.20[b]	22.76 ± 0.71[bc]	103.53 ± 1.18[a]
	400 W + 45°C	39.30 ± 0.32[b]	−5.73 ± 0.01[b]	23.97 ± 0.26[a]	24.61 ± 0.37[b]	103.52 ± 0.07[a]
	600 W + 45°C	40.31 ± 0.16[a]	−6.67 ± 0.04[c]	21.04 ± 0.12[c]	27.11 ± 0.12[a]	103.85 ± 0.13[a]
F-test	*	*	*	*	*	*
Red	45°C	40.26 ± 0.87[b]	−3.61 ± 0.16[a]	23.98 ± 0.06[b]	24.44 ± 0.07[c]	99.17 ± 0.87[c]
	200 W + 45°C	40.05 ± 0.81[b]	−4.82 ± 0.05[b]	23.21 ± 0.18[c]	23.60 ± 0.18[d]	101.83 ± 0.06[b]
	400 W + 45°C	40.70 ± 0.33[b]	−5.42 ± 0.03[c]	24.92 ± 0.21[a]	25.44 ± 0.21[b]	102.35 ± 0.02[a]
	600 W + 45°C	43.24 ± 0.14[a]	−5.95 ± 0.01[d]	21.99 ± 0.29[d]	26.53 ± 0.29[a]	102.26 ± 0.05[a]
F-test	*	*	*	*	*	*

Values are represented as mean ± SE ($n = 4$). Different letters indicate significant differences between lines at $p < 0.05$ according to Duncan's Multiple Range Test (DMRT) at a 95% significant difference. *Significant difference at $p < 0.05$.

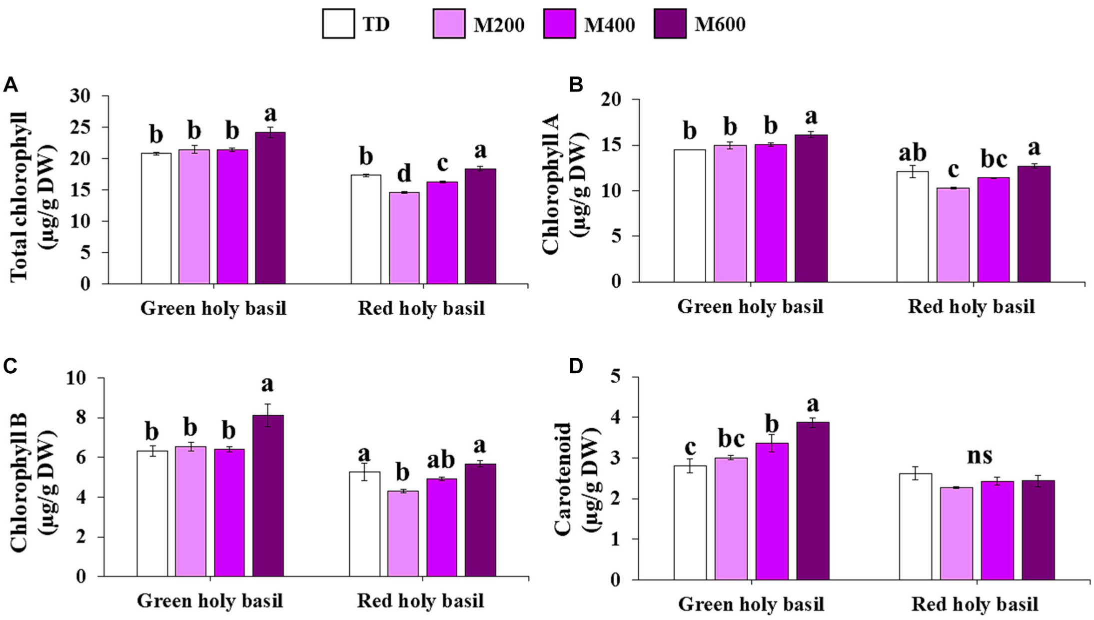

FIGURE 4
Pigment accumulation, total chlorophyll **(A)**, Chl a **(B)**, Chl b **(C)**, and carotenoid **(D)** in green and red holy basil leaves after tray drying (TD, 45°C) and drying under various microwave power combinations with hot air at 45°C (HAD) (200, 400, and 600 W). ANOVA was performed, followed by mean comparisons with DMRT. The different letters above the bars show significant differences between treatments at $p < 0.05$. "ns" indicates no significant difference.

temperature gradient between the sample and the air in the drying chamber that promotes the moisture in the samples to move outwards (Hemis et al., 2011; Doymaz et al., 2015; Hou et al., 2021; Mouhoubi et al., 2022). Previous reports of the Trabzon persimmon fruit (Alibas et al., 2019) and Kaffir lime leaf (Pradechboon et al., 2022) showed that increases in MD power induced a rapid increase in temperature due to the high energy transfer into samples. Our study indicates that MD at 600 W with HAD treatment ensured the shortest drying time for both green and red holy basil leaves of 3 and 2.5 h, respectively. In comparison, holy basil leaves dried by tray drying (TD) showed the longest drying time at 7 and 6 h for green and red holy basil leaves, respectively. This result was consistent with previous reports showing that an increase in MD power causes a dramatic increase in the average drying rates of various plants (Alibas et al., 2019; Liu et al., 2021; Pradechboon et al., 2022).

Energy consumption values among different drying methods of red and green holy basil leaves are shown in Table 2. MD methods had high energy-saving effects when compared with TD, which is widely used in chemical, pharmaceutical, and food industries to preserve herbal products for downstream processing and extended storage (Mujumdar, 2008; Chen and Mujumdar, 2009). Our study shows that all MD treatments have a lower and specific energy consumption (SEC) than TD. Further, microwave drying at 600 W with HAD provided around 2–3-fold energy consumption reduction over TD for

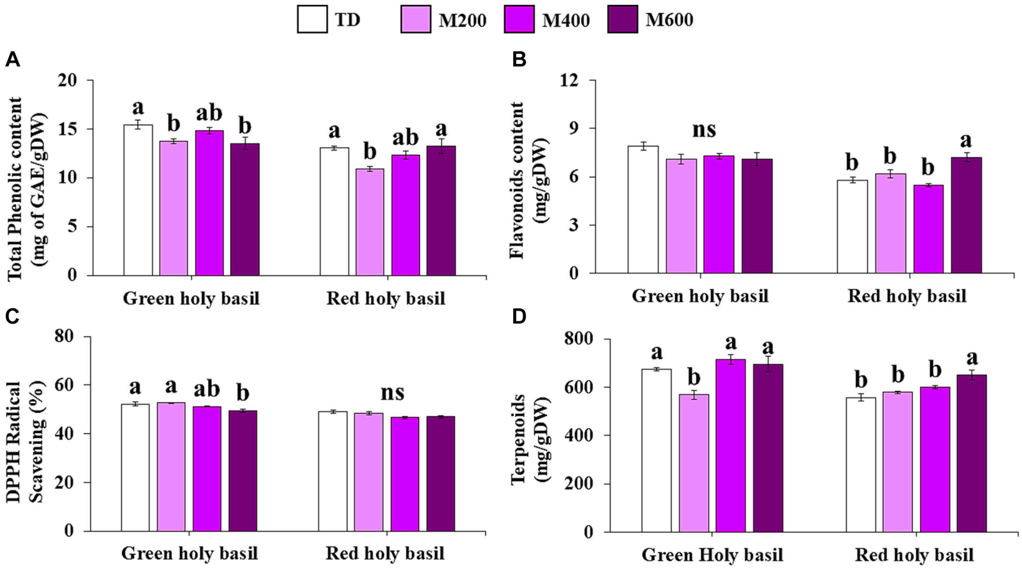

FIGURE 5
The content of total phenolic content (TPC) **(A)**, total flavonoid content (TFC) **(B)**, total terpenoids content **(C)**, and DPPH free radical scavenging rate **(D)** in green and red holy basil leaves after tray drying (TD, 45°C) and various microwave power combinations with hot air at 45°C (HAD) (200, 400, and 600 W). ANOVA was performed, followed by mean comparison with DMRT. The different letters above the bars show significant difference between treatments at $p < 0.05$. "ns" indicates no significant difference.

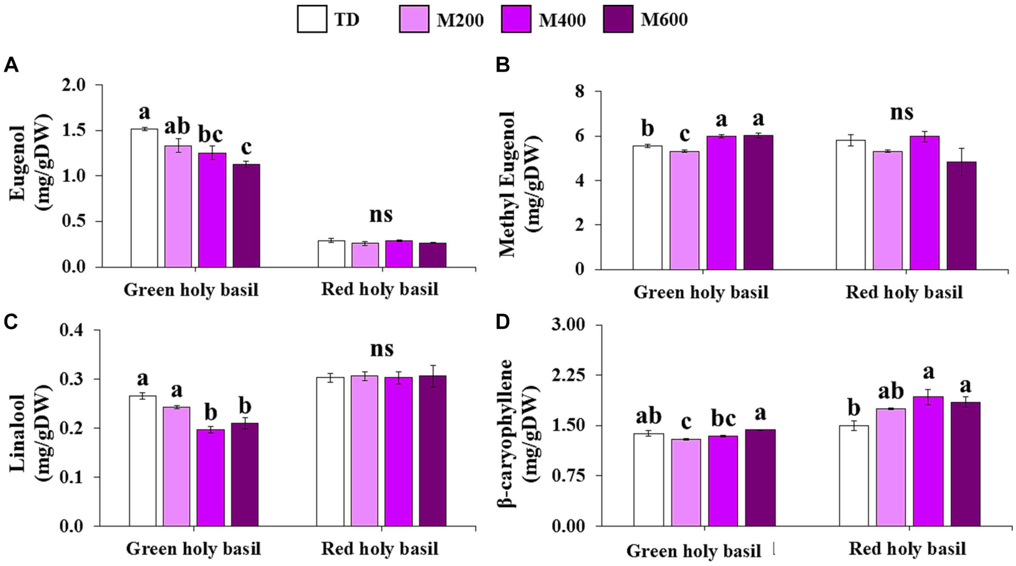

FIGURE 6
The accumulation of eugenol **(A)**, methyl eugenol **(B)**, Linalool **(C)**, and β-caryophyllene **(D)** in green and red holy basil leaves after tray drying (TD, 45°C) and various microwave power combinations with hot air at 45°C (HAD) (200, 400, and 600 W). ANOVA was performed, followed by mean comparison with DMRT. The different letters above the bars show a significant difference between treatments at $p < 0.05$. "ns" indicates no significant difference.

green and red holy basil leaves, respectively. The increase in microwave power enhanced the movement of water molecules since microwaves can easily penetrate inside the sample, where the microwave energy is transformed into heat. When the molecules of water are rubbed together, they produce heat, which increases the water temperature. In the case of TD, the heat is transferred from the surface of the sample to inside it by heat conduction, causing a longer drying time (Chen et al., 2019). These results are in accordance with previous findings that energy consumption and SEC decrease as microwave power increases (Varith et al., 2007; Kaveh et al., 2021). This is largely due to the drying time being shorter. Moreover, these results suggest that the highest MD power (600 W with HAD) might increase evaporation rates during the drying process, leading to decreases in energy consumption.

Drying processes can significantly affect the qualitative features of herbal production. Color is a psychological property of food products that directly influences the perception of flavor and quality (García et al., 2009), and the drying process is one of the most important causes of color degradation or dehydration (Lozano and Ibarz, 1997; Demiray et al., 2017). This is likely due to the gradual rising of heat, leading to the decomposition of colored pigments (Orikasa et al., 2014). Studies in various plant leaves and fruits have shown that the temperature during the drying process results in color changes in spearmint leaf (Islam et al., 2019), holy basil leaf (Raksakantong et al., 2011), Kaffir lime leaf (Pradechboon et al., 2022), papaya (Islam et al., 2019), and kiwi (Izli et al., 2017; Taghinezhad et al., 2021).

Likewise, in the current study, we observed that the drying treatment significantly influenced color change in the dried leaf powders of green and red cultivars. Colorimetric parameters, such as *L**, *a**, *b**, and *C*, are widely used to quantify the coloration of food and fruit products (Arias et al., 2000; Pathare et al., 2013). *L** represents lightness, *a** represents red (positive value) and green (negative value), *b** represents yellow (positive value), and blue (negative value), and *C* represents color saturation. H represents relative amounts of redness and yellowness, with 90° for yellow, 180° for green, and 270° for blue (Francis, 1980; Voss, 1992; Ayala-Silva et al., 2005).

Our results showed that 600 W of MD with HAD caused the highest values of *L**, *a**, *C*, and *h* (hue) of green and red holy basils with *b** at its lowest when compared with other MD treatments and conventional TD (Table 3). This is consistent with previous studies that found MD (increased power) with HAD caused low *a** values (Yilmaz and Alibas, 2021; Pradechboon et al., 2022). However, there were no significant differences between the L* and b* values of green holy basil at 600 W of MD with HAD and under TD conditions. This may be explained by the drying time of TD, which, without a microwave, took longer time to dry than 600 W of MD with HAD. The TD condition had lower heat than microwave power and resulted in brightness and yellow shades that were similar to 600 W of MD with HAD treatment. Similarly, the leaf pigment contents of the dried samples of green and red holy basils showed the highest accumulation of total chlorophyll (TChl), chlorophyll a (Chl a), and chlorophyll b (Chl b) in MD 600 W (Figure 4). These findings indicate that MD 600 W with HAD treatment, with its shortest drying duration, is promising in terms of psychologically interpreted properties of herbal products by maintaining greener and lighter colors in dried holy basil leaves of both cultivars.

Secondary metabolites play an important role in plant defense response and are widely used to relieve several ailments in traditional medicine (Afzal et al., 2022; Aziz et al., 2022; Kainat et al., 2022; Khalid et al., 2022). Essential oils, terpenes, phenolics, phenolic acids, and flavonoid compounds are the main constituents of antioxidants in the holy basil plant (Kadian and Parle, 2012; Agarwal et al., 2017; Maqbool et al., 2023; Waliat et al., 2023). The drying process may also induce changes in the secondary metabolites, bioactive compounds, and volatile oils among herbal products. Several studies have demonstrated that high temperature seriously damages secondary plant metabolites such as total phenolic compounds (TPC), total flavonoid compounds (TFC), terpenoids, antioxidant properties, and volatile oils in various plant species (Sang et al., 2014; Hihat et al., 2017; Nguyen and Le, 2018). Phenolic compounds and flavonoids are the most important phytochemical components and have potential against diseases, presumably due to their antioxidant properties (Rice-Evans, 2001). In our study, after drying the green holy basil leaves, levels of TPC, DPPH, and terpenoids were highest at 400 W MD and similar to that of TD treatment. In contrast, red holy basil leaves showed the highest of these secondary metabolites and antioxidants at 600 W of MD with HAD. However, the highest power of MD caused degradation in secondary metabolites and antioxidants in green holy basil leaves, whereas red holy basil leaves were not significantly affected by the highest power of MD. This result is consistent with previous reports that found that phenolics and flavonoids decreased at higher microwave powers in herbal leaves and cereal grains (Xiao et al., 2008; Ragaee et al., 2014; Thiangma et al., 2022; Zia et al., 2022). This may be because high microwave power levels beyond a threshold limit cause oxidation and thermal degradation of aromatic compounds by enzyme inactivation, causing decreases in bioactive compound levels (Nüchter et al., 2004; Thiangma et al., 2022). Optimization of drying and operating conditions mainly depends on the characteristics of plant raw materials as well as processing methods. This necessitates that the drying conditions be chosen based on the type of herbal material. Our findings clearly indicate that dried leaves of green holy basil with the highest TPC, terpenoids, and antioxidant contents were found at 400 W of MD with HAD.

The major components among volatile organic compounds (VOCs) of holy basil are phenylpropanoids and terpenoids (Chutimanukul et al., 2022a,b). Several target compounds have shown beneficial impacts on humans, including antioxidant properties, antibiotic-resistant bacteria, and antifungal and anti-inflammatory activities (Burt, 2004; Pandey et al., 2017). The target compounds required by food, pharmaceutical, and cosmetic industries are methyl eugenol, eugenol, Linalool, and β-caryophyllene content, which are the primary VOC compounds produced in holy basil (Vyas et al., 2014; Chutimanukul et al., 2022b). Eugenol and methyl eugenol are widely used for flavoring foods and have been extensively used for their anti-inflammatory, antioxidant, antibacterial, and analgesic properties (De Vincenzi et al., 2000; Choi et al., 2010; Pramod et al., 2010; Nejad et al., 2017). Linalool is a monoterpene that occurs naturally in aromatic plants and is commonly used as a chemical ingredient for flavor and fragrance in cosmetics (Arctander, 1969; Villa et al., 2007; Kamatou and Viljoen, 2008). β-caryophyllene is the primary sesquiterpene used in medicinal applications due to its anticancer (Fidyt et al., 2016), diabetes (Basha and Sankaranarayanan, 2016), colitis (Dutra et al., 2011), anxiety, and depression properties (Bahi et al., 2014).

Our study showed that VOC content was influenced by drying variables, particularly in green holy basil (Figure 6). For green holy basil leaves, raising the power of MD decreased eugenol and linalool content (Figures 6A,C). However, raising the MD power from 200 to 400 W increased the content of methyl eugenol and β-caryophyllene, especially at the maximum power of MD 600 W (Figures 6B,D). For red holy basil, the drying treatment did not significantly affect the content of eugenol, methyl eugenol, and Linalool. Interestingly, increasing MD power caused higher β-caryophyllene content in red holy basil leaves (Figure 6). This was consistent with previous reports (Harborne et al., 1993) that examined the effect of different drying processes on the yield of volatile products in plants and vegetables. The physicochemical changes of volatile compounds during the drying process depend on several factors, such as the drying method, post-harvest practice, and the instability of essential properties (Venskutonis, 1997; Thamkaew et al., 2021). One of the most important chemical alterations of VOCs is oxidation and isomerization, which are deterioration processes of secondary products either enzymatically or chemically (Turek and Stintzing, 2013). Moreover, heat promotes the initial formation of free radicals, which catalyzes the autoxidation reaction (Lee et al., 2007). These results suggest the highest power at 600 W of MD with HAD condition could maintain the methyl eugenol and β-caryophyllene content in green holy basil leaves, while 400 and 600 W of MD could induce higher contents of β-caryophyllene in dried leaves of red holy basil. This finding should be of great value in fine-tuning the drying processes of holy basil in terms of food quality preservation and product development. Notably, the application of MD power with the HAD method could be used to produce high-value products while maintaining desired characteristics for the food and cosmetic industries.

Conclusion

Our study has established that the use of microwave drying (MD) combined with hot air drying at 45°C (HAD) can be successfully used to preserve the quality, bioactive compounds, and volatile organic compounds (VOCs) of the two cultivars of holy basil leaf for food and pharmaceutical industries. Our data clearly indicates that MD power at 600 W with HAD has the highest ability to maintain color change of leaf in both cultivars by decreasing chlorophyll and carotenoid content. While 400 W of MD with HAD can be proposed for stabilization of TPC and DPPH free radical scavenging activity in green holy bail leaves, these bioactive compounds in red cultivars had the highest accumulation at 600 W of MD treatment. Moreover, 600 W of MD with HAD also exhibited the highest VOC content comprising methyl eugenol and β-caryophyllene for both cultivars. Based on the findings of our study, using 600 W of MD proved to be highly efficient in terms of energy savings resulting from significantly shortened drying times. This alternative method of drying shows promise as a viable option for green and red holy basils and could potentially replace conventional drying methods. The adoption of these alternatives could lead to better product qualities and higher bioactive compound retention, as well as lower electrical energy consumption at industrial scales.

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

PC, TT, and TS: study conception and design. LW, HJ, AT, and PC: data collection. LW, NP-a, KM, and PC: analysis and interpretation of results. LW and PC: writing the original draft. CD: revise the manuscript. PC and CD: writing review and editing. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

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

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Mechanism of enhanced freshness formulation in optimizing antioxidant retention of gold kiwifruit (*Actinidia chinensis*) harvested at two maturity stages

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Kiwifruit exhibits a climacteric ripening pattern and has as an extremely perishable nature. Considering that high perishability leads to a loss in antioxidants and overall nutritional quality. This study aimed to examine the efficacy of enhanced freshness formulation (EFF), a hexanal-based formulation containing antioxidants such as geraniol, α-tocopherol and ascorbic acid, on maintaining the bioactive compounds of gold kiwifruit (cv. 'Y368') harvested at two maturity stages. Kiwifruits were treated with three treatments, namely, control (untreated fruit), 0.01 and 0.02% (v/v) EFF. Fruits were treated with 8 weeks of cold storage at 0°C and 90% relative humidity, then transferred to 20°C for 8 days. Three bioactive compounds (ascorbic acid, total phenolics and flavonoids), antioxidant capacities using DPPH and FRAP assays, polyphenol oxidase, ascorbate oxidase, phenylalanine and tyrosine ammonia lyase enzyme activities were evaluated. The results showed that EFF significantly ($p < 0.05$) influenced bioactive compounds, antioxidant capacities and the activity of enzymes involved in the synthesis and oxidation of bioactive compounds. The maturity stage significantly influenced the content of bioactive compounds. Later harvested kiwifruit had greater content of bioactive compounds, compared to earlier harvested kiwifruit. The total phenolic content was 0.77, 1.09 and 1.22 mg GAE g^{-1} FW for control, 0.01 and 0.02% EFF, respectively. The FRAP antioxidant concentration was 0.76, 0.91 and 0.96 μmol Fe (II) g^{-1} FW for control, 0.01 and 0.02% EFF. The findings illustrate the capacity of EFF to optimize bioactive compounds and storability of kiwifruit during postharvest storage.

KEYWORDS

hexanal, postharvest quality, maturation, antioxidant capacity, gold kiwifruit

Introduction

The action of bioactivities including ascorbic acid, carotenoids and various flavanones and polyphenols contribute to the health beneficial properties of kiwifruit (Pérez-Burillo et al., 2018). Gold (yellow-fleshed) kiwifruit (*Actinidia chinensis*) possesses significant nutritional value and an array of bioactive compounds which make it a good source of antioxidants. The high nutritional value of kiwifruit has resulted in an increasing global demand for kiwifruits

(Ma et al., 2017). According to the Post-Harvest Innovation (PHI) Programme, it takes approximately 25 days to export South African fresh produce to Europe. However, deterioration of logistical infrastructure and shortage of refrigerated containers causes congestion and shipping delays at South Africa's ports (Brodie, 2022). In addition to this, the delivery of kiwifruit exported by sea is delayed by at least three weeks, due to delays that transpire at the European port (Eichstaedt, 2022).

Delays experienced along the supply chain result in extended storage duration of kiwifruit. These challenges present a problem since several kiwifruit cultivars have an extremely perishable nature due to their high metabolic activity (Asiche et al., 2017). This leads to a significant loss in bioactive compounds of kiwifruit (Huang et al., 2017; Cha et al., 2019; Kumarihami et al., 2020). The kiwifruit industry highly relies on cold storage, which facilitates in prolonging the storage of kiwifruit (Shin et al., 2018; Cha et al., 2019; Xia et al., 2020). However, cold storage alone is not enough to optimize quality if the storage duration is prolonged. Findings from previous research have illustrated that prolonged storage under cold conditions (0–5°C) results in a significant loss of bioactive compounds of kiwifruit (Goffi et al., 2019; Jeong et al., 2020; Xia et al., 2020; Choi et al., 2022). The importance of incorporating postharvest treatments with cold storage to reduce the loss of bioactive compounds is well established in kiwifruit.

High demand from European markets necessitates South African growers and exporters to develop strategies for preserving kiwifruit during extended storage periods, aiming to meet the increasing demand for high-quality kiwifruit with health-promoting characteristics. Fruit maturity is an essential factor that influences the concentration of bioactive compounds and the postharvest performance of kiwifruit (Deepthi and Sekhar, 2015; Lee et al., 2015; Nkonyane et al., 2022). Literature has demonstrated that kiwifruit should be harvested when the total soluble solid content is at a minimum of 6.2 °Brix, in order to optimize fruit quality during storage (Burdon et al., 2016). Mahlaba et al. (2021) also determined that a minimum harvest and export value should be ≥6.2 °Brix for gold kiwifruit produced in South Africa. Thus, harvesting at the optimum maturity stage minimizes the loss of bioactive compounds. In spite of this, South African gold kiwifruits destined for export, are not harvested at a singular maturity stage but harvested at the mid (≥ 6.2 °Brix) and late (≥ 8.5 °Brix) maturity stages (Mahlaba et al., 2021; Nkonyane et al., 2022). However, these authors demonstrated that late harvested kiwifruit experience a substantial decrease in quality during storage. Therefore, it is essential to employ postharvest treatments that can maintain bioactive compounds of kiwifruit harvested at various maturity stages.

Past research has investigated the impact of various postharvest treatments such as 1-methylcyclopropene (Lim et al., 2016; Xu et al., 2021; Zhang et al., 2021), edible coatings (Allegra et al., 2016; Hu et al., 2019; Kumarihami et al., 2022), ozone (Goffi et al., 2019; Wang et al., 2022), UV-C (Hu et al., 2022) and heat treatment (Shahkoomahally and Ramezanian, 2015; Chiabrando et al., 2018) on the maintenance of bioactive compounds in kiwifruit. Notably, these studies predominantly focused on kiwifruit harvested at only one maturity stage. To the best of our ability, we have not found studies investigating the efficacy of postharvest treatments on optimizing bioactive compounds of gold kiwifruit harvested at different maturity stages.

Considering this gap in literature and the growing demand for high-quality kiwifruit, it is essential to explore a postharvest treatment that can optimize the bioactive compounds of kiwifruit harvested at various maturity stages. Hexanal is a naturally occurring volatile compound, specifically a six-carbon aldehyde formed from linoleic acid via the lipoxygenase pathway during lipid peroxidation in plants (Paliyath and Padmanabhan, 2018). Enhanced freshness formulation (EFF) is a formulation that comprises of 1% hexanal, 1% geraniol, 1% α-tocopherol and 1% ascorbic acid (Cheema et al., 2014). EFF has the capacity to optimize phenolics and ascorbic acid of various fruit (Cheema et al., 2014; Gill et al., 2016; Jincy et al., 2017). Hexanal's capacity to reduce the loss in bioactive compounds could serve as a potential tool of optimizing quality and storability of South African grown gold kiwifruit. South African gold kiwifruits are harvested at both the mid and late maturity stage. However, literature has illustrated that late harvested experience a substantial decrease in quality. Considering that the export of South African grown gold kiwifruit is expected to rise given the growing demand from international markets, this makes it necessary to devise a postharvest strategy that can maintain optimum quality of kiwifruit harvested at both maturity stages.

Therefore, the current study was conducted to evaluate the efficacy of EFF to optimize bioactive compounds (phenolic and flavonoid compounds, ascorbic acid and carotenoids) of gold kiwifruit (cv. 'Y368') harvested at two maturity stages. Furthermore, the activity of enzymes, phenylalanine ammonia-lyase, tyrosine ammonia-lyase, polyphenol oxidase and ascorbate oxidase, involved in the synthesis and oxidation of these bioactive compounds (phenolic and flavonoid compounds, ascorbic acid and carotenoids) were investigated.

Materials and methods

Materials

Kiwifruit (*Actinidia chinensis*) cv. "Y368" were harvested from Roselands farm, a commercial kiwifruit farm located in the Richmond area (Latitude: 29.9033°S, Longitude: 30.2397°E), KwaZulu-Natal Province, South Africa. Kiwifruits were harvested when the total soluble solid content was greater than 6.2 °Brix (Mahlaba et al., 2021), at two maturity stages namely, maturity stage one (M1) and maturity stage two (M2) with 7 and 9 °Brix, respectively. Harvested fruit were immediately transported in a ventilated vehicle to the Postharvest Laboratory of the University of KwaZulu-Natal, where kiwifruit without blemishes, decay or physical damage were graded and selected for uniformity in size, then assigned to the respective postharvest treatments. All chemicals used to conduct the experiments in this study were purchased from Sigma-Aldrich, South Africa.

Preparation of postharvest treatments and storage

The enhanced freshness formulation (EFF) was prepared by making a stock formulation comprising of 1% (v/v) hexanal, 1% (v/v) geraniol, 1% (w/v) α-tocopherol, 1% (w/v) ascorbic acid, 0.1% (w/v) cinnamic acid and 10% (v/v) Tween 20 dissolved in ethanol (10% v/v) (Cheema et al., 2014). A 0.01 and 0.02% (v/v) hexanal concentrations were prepared by mixing the stock solution in 100 L and 50 L of distilled water, respectively. Kiwifruit were immersed in 0.01 and 0.02% (v/v) EFF solution for 2.5 min, then air dried at room temperature (Cheema et al., 2014). Control fruit were left untreated. Each treatment consisted of three replicates (n = 3); each replicate had three fruit per storage interval for each maturity stage. The kiwifruits were stored for 8 weeks in a cold room with temperature set at 0°C and relative humidity at 90%, then transferred to 20°C for one week to stimulate shelf life.

Total phenolic content

The total phenolic content was determined using a method described by Singleton et al. (1999). Kiwifruit pulp (1 g) was homogenized with 5 mL of 80% methanol. Thereafter, 2.6 mL of distilled water and 200 μL of Folin–Ciocalteu's phenol reagent was added to 200 μL of the methanolic extracts. After 6 min, 2 mL of 7% (w/v) sodium carbonate was added to the reaction mixture. The absorbance was measured at 750 nm after 90 min using a Shimadzu UV spectrophotometer (Model UV-1800 240 V, Kyoto, Japan). The total phenolic content was expressed as mg gallic acid equivalents GAE g^{-1} fresh weight (FW) of kiwifruit.

Total flavonoid content

Distilled water (3.2 mL) and 150 μL of 5% (w/v) sodium nitrite was added to 500 μL of methanolic extract. After 5 min, 150 μL of 10% (w/v) aluminum chloride was added. After 6 min, 1 mL of 1 M sodium hydroxide was added and the absorbance recorded at 510 nm. The total flavonoid content was expressed as mg catechin equivalents CE g^{-1} fresh FW of kiwifruit.

Ascorbic acid

A method described by Malik and Singh (2005) and modified by Goffi et al. (2019), was used to determine the ascorbic acid content of kiwifruit. Ascorbic acid was extracted from 1 g of kiwifruit pulp using 4 mL of 16% (v/v) metaphosphoric acid, containing 0.18% (w/v) disodium ethylenediaminetetraacetic acid and centrifuged at 1100 × g for 10 min at 4°C. The assay mixture contained 200 μL supernatant, Folin's reagent (1: 5 v/v) and 0.3% (v/v) metaphosphoric acid in a final volume of 2 mL. The absorbance was measured at 760 nm, using ascorbic acid as standard and expressed as mg g^{-1} FW.

Total carotenoids

Total carotenoid content was determined using a method adapted from Yan et al. (2015) and modified by Xia et al. (2020). Five milli liter of acetone containing 0.1% butylated hydroxytoluene was used to extract carotenoids from 1 g of kiwifruit pulp. After centrifugation at 10,000 × g for 10 min at 4°C, the supernatant was filtered through the Whatman filter paper No. 42. The total carotenoid content was determined by measuring the absorbance of the extract at 450 nm and expressed as μg g^{-1} FW.

DPPH radical-scavenging activity

DPPH was assessed using a protocol described by Brand-Williams et al. (1995). Thus, 6×10^{-5} mol/L DPPH was dissolved in methanol (1 L) and 3.9 mL of this solution was added to 100 μL of methanolic extracts. After 30 min at room temperature, the absorbance of the reaction mixture was measured at 517 nm. The DPPH radical scavenging activity was quantified using Eq. (1) and expressed as percentage DPPH radical-scavenging activity (DPPH %)

$$(\%) = \left[Absorbance(control) - Absorbance(sample) \right] / \left(Absorbance(control) \right) \times 100 \quad (1)$$

Ferric-reducing antioxidant power

The FRAP was assessed using a previously reported protocol (Benzie and Strain, 1996). The FRAP working solution was prepared daily by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$ solution at a ratio of 10:1:1. The freshly solution was warmed at 30°C before usage. Then, 100 μL of each kiwifruit pulp extract was mixed with 3 mL of FRAP solution and incubated at 37°C for 4 min. Thereafter, the absorbance was recorded at 593 nm. A calibration curve was prepared using ferrous sulfate. The findings were expressed as μM g^{-1} FW.

Extracts for phenylalanine ammonia lyase and tyrosine ammonia lyase

The enzymatic activity of PAL (EC 4.3.1.5) and TAL (EC 4.3.1.25) was evaluated using a method described by Khan et al. (2003). Extraction was carried out by homogenizing 1 g of kiwifruit pulp with 10 mL of ice-cool Tris–HCl buffer (50 mM, pH 8.5) comprising of 5% (w/v) polyvinylpolypyrrolidone (PVPP) and 14.4 mM β-mercaptoethanol. After centrifugation at 10000 g at 4°C for 20 min, the enzyme activity of PAL and TAL was quantified using the recovered supernatant. Bradford assay Bradford (1976) was used to determine the protein concentration.

Phenylalanine ammonia lyase

The activity of PAL was quantified using an assay mixture containing 800 μL of Tris–HCl buffer (0.5 mM, pH 8.0), 100 μL 6 μM of L-phenylalanine, and 100 μL of enzyme extract. After incubation at 40°C for an hour, the reaction was stopped by adding 100 μL of 5 N HCl to the mixture. The enzyme activity was quantified using the absorbance measured at 290 nm. The activity was expressed as U g^{-1} of protein, where a unit of enzyme activity was defined as the amount of enzyme that produces 1 nmole of cinnamic acid min^{-1}.

Tyrosine ammonia lyase

The activity of TAL was quantified using an assay mixture containing 800 μL of Tris–HCl buffer (0.5 mM, pH 8.0), 100 μL of 5.5 μM of L-tyrosine and 100 μL of enzyme extract. After incubation at 40°C for an hour, the reaction was stopped by adding 100 μL of 5 N HCl to the mixture. The enzyme activity was quantified using the absorbance measured at 333 nm. The activity was expressed as U g^{-1} of protein, where a unit of enzyme activity was defined as the amount of enzyme that produces 1 nmole of coumaric acid min^{-1}.

Ascorbate oxidase

The enzyme activity of ascorbate oxidase (EC 1.10.3.3) was assayed using a previously described protocol by Jiang et al. (2018). AO was extracted by homogenizing 1 g of pulp with 3 mL potassium phosphate buffer (50 mM, pH 7.5) comprising of 1 mL of potassium phosphate buffer (100 mM, pH 6.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.3% (w/v) Triton X-100, 4% (w/v) polyvinyl-polypyrrolidone (PVPP), and 0.1 mM ascorbic acid. After centrifugation at 16000 g for 15 min, the absorbance was measured. The enzyme activity was expressed as U g^{-1} of protein and defined as the amount of enzyme causing an increase of 0.01 in the absorbance per min at 265 nm at 25°C. The Bradford assay Bradford (1976) was used to determine the protein concentration.

Polyphenol oxidase activity

A protocol by Adiletta et al. (2019) was used to assay PPO (EC 1.14.18.1) activity. One gram of pulp was homogenized in sodium phosphate buffer (100 mM, pH 6.4) comprising of 0.05 g of PVPP. After centrifugation at 10,000 g for 30 min at 4°C, 100 µL of the enzyme extract was added to a buffered substrate (500 mM catechol in 100 mM sodium phosphate buffer, pH 6.4) and the increase in absorbance was monitored at 398 nm. PPO activity was expressed as U g^{-1} of protein. The Bradford assay Bradford (1976) was used to determine the protein concentration.

Statistical analysis

All experiments were performed using a completely randomized factorial design. The experiment comprised of three factors: treatments, fruit maturity stage and storage period. Statistical analysis was performed in R software version 4.3.1 (R Core Team, 2023). Data were expressed as mean values ± SE and were subjected to analysis of variance (ANOVA) with a 5% level of significance. A Pearson correlation test was used to correlate the measured parameters.

Results and discussion

Total phenolic and flavonoid content

The interaction among treatment, maturity stage, and storage time had no significant ($p > 0.05$) effect on the TPC. The results illustrate that treatments significantly influenced ($p < 0.001$) the TPC (Figure 1A), with treated fruit registering substantially greater TPC compared to the control. The TPC was 0.88, 1.17 and 1.29 mg GAE g^{-1} FW for the control, 0.01 and the 0.02% EFF treatment level, respectively. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on TPC. However, the interaction between maturity stage and storage time (Figure 1B) had a significant ($p < 0.001$) effect on TPC, with M2 registering significantly higher TPC than M1. Kiwifruit at both maturity stages exhibited an increase in TPC, reaching maximum values of 1.21, and 1.58 mg GAE g^{-1} FW for M1 and M2, respectively, at six weeks of storage. At the end of storage, TPC for M1 and M2 was 1.06 and 1.19 mg GAE g^{-1} FW, respectively.

The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on the TFC. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on TFC. However, the combined effect of treatment and maturity stage (Figure 1C) significantly ($p < 0.001$) influenced the TFC. Treated fruit exhibited significantly higher TFC compared to the control. Maturity stage influenced the TFC, with M2 exhibiting significantly greater TFC relative to M1. Significant differences among treated fruit were only observed at M2, with 0.02% EFF registering higher TFC. At M1, no significant differences among treated fruit were observed. In addition, the TFC significantly ($p < 0.001$) increased throughout the storage period (Figure 1D).

The findings of this study illustrated that storage duration affects phenolic content of Y368 kiwifruit. This is characterized by a significant decrease in TPC after six weeks of storage. These findings corroborate those of Lim and Eom (2018), Goffi et al. (2019), and Jeong et al. (2020), who demonstrated that TPC in kiwifruit decreased during cold storage. Furthermore, Gullo et al. (2016) and Nkonyane et al. (2022), attained comparable findings to the present study. These authors illustrated that kiwifruit exhibited an increase in TPC when transferred to ambient conditions. Results obtained from this study demonstrated that EFF-treated kiwifruit had substantially higher TPC and TFC during storage. The findings attained are in alignment to those reported by Gill et al. (2016), Sharma et al. (2023), and Öz et al. (2023), who demonstrated that EFF effectively maintained higher phenolic content of guava, jujube and persimmon fruit, respectively. Secondary metabolites such as phenolic and flavonoid compounds are crucial for the maintenance of fruit quality, as they delay senescence induced by oxidative degradation, by functioning as antioxidants that scavenge free radicals (Gulcin, 2020). Therefore, the efficacy of EFF to enhance TPC and TFC may contribute to optimized quality and storability of kiwifruit during storage.

Ascorbic acid

The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on AA. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on AA. The treatments had a significant effect ($p < 0.001$) on AA content (Figure 1E), with the 0.02% treated fruit registering higher AA content, followed by 0.01% treated fruit. AA content for the control, 0.01 and 0.02% EFF treatment level was 2.72, 3.12 and 3.34 mg g^{-1} FW. These findings indicate that EFF treatment application effectively retained AA content throughout the storage period. Our results corroborate those reported by Cheema et al. (2014), Gill et al. (2016), Jincy et al. (2017), Silué et al. (2022) and Sharma et al. (2023) who illustrated that EFF retained ascorbic acid of tomato, guava, mango and jujube fruit, respectively. The maturity stage had a significant effect ($p < 0.001$) on AA content (Figure 1F), with M2 (3.53 mg g^{-1} FW) exhibiting a greater AA content than M1 (2.54 mg g^{-1} FW).

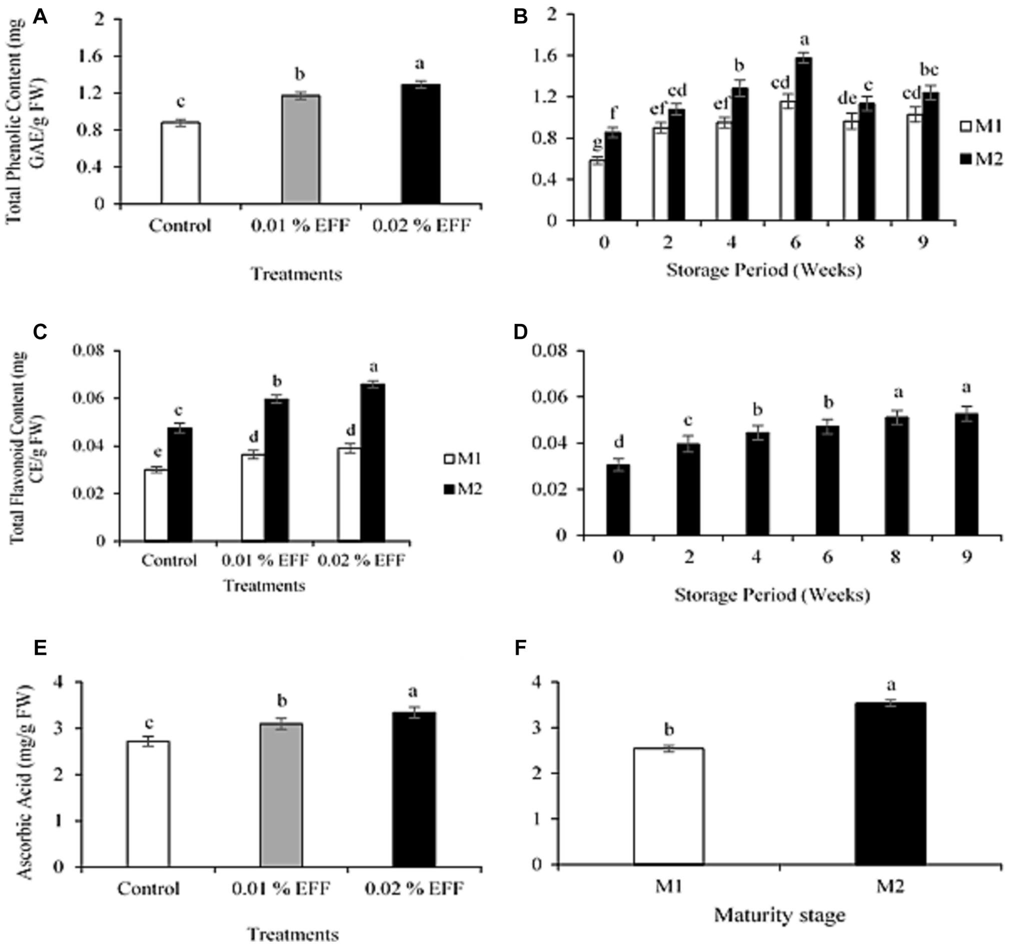

FIGURE 1
Effect of treatments **(A)** and the effect of interaction between maturity stage and storage time **(B)** on the total phenolic content of kiwifruit. Effect of interaction between treatment and maturity stage **(C)** and the effect of storage time **(D)** on the total flavonoid content of kiwifruit. Effect of treatments **(E)** an maturity stage **(F)** on ascorbic acid content of kiwi fruit. Values are the means ± SE (*n* = 3).

Findings from the present study are in alignment with those of Lim and Eom (2018), who illustrated that AA content increased with an advancement in maturity. In plants, AA preserves cell integrity by reducing hydrogen peroxide and reacting rapidly with radical species (Dumanović et al., 2021). Thus, it provides DNA and lipids with protection from oxidative damage (Meitha et al., 2020). The loss of AA in kiwifruit during storage is estimated to be between 50 and 70% (Sharma et al., 2015). AA undergoes oxidative loss as it is converted to dehydroascorbic acid by the enzymatic action of ascorbate oxidase (Chatzopoulou et al., 2020). EFF has been reported to delay the loss of AA in guava fruit by reducing the oxidation process (Gill et al., 2016). Therefore, the findings from the present study show that EFF has the potential to preserve the nutritional value of kiwifruit by delaying the loss of AA during storage.

Total carotenoids

The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on TC. Treated kiwifruit exhibited significantly ($p < 0.01$) lower TC, compared to the control (Figure 2A). The TC was registered at 5, 4.78 and 4.71 μg g^{-1} FW for the control, 0.01 and 0.02% treatment level, respectively. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on TC. The interaction between maturity stage and storage period significantly ($p < 0.05$) influenced the TC content (Figure 2B). The TC significantly decreased after four weeks of storage for kiwifruit harvested at M1. TC significantly decreased after two weeks for kiwifruit harvested at M2. Throughout the storage period, kiwifruit harvested at M2 exhibited greater TC relative to M1.

The antioxidant properties of carotenoids provide protection from certain kinds of cancer. EFF treated fruit exhibited less carotenoid content relative to the untreated kiwifruit. Similarly, Tiwari and Paliyath (2011) and Dek et al. (2018) showed that EFF lowered the accumulation of carotenoids in tomato fruit. This may be indicative of better-quality maintenance due to a slower rate of ripening (Cheema et al., 2018). Storage temperature has a strong influence on carotenoid metabolism (Matsumoto et al., 2009). Exposing kiwifruit to low temperatures slows down the accumulation of carotenoids, whereas high temperatures enhance the accumulation of carotenoids (Xia et al., 2020). Low storage temperatures upregulate the expression of cleavage dioxygenase and 9-cis-epoxycarotenoid dioxygenase involved

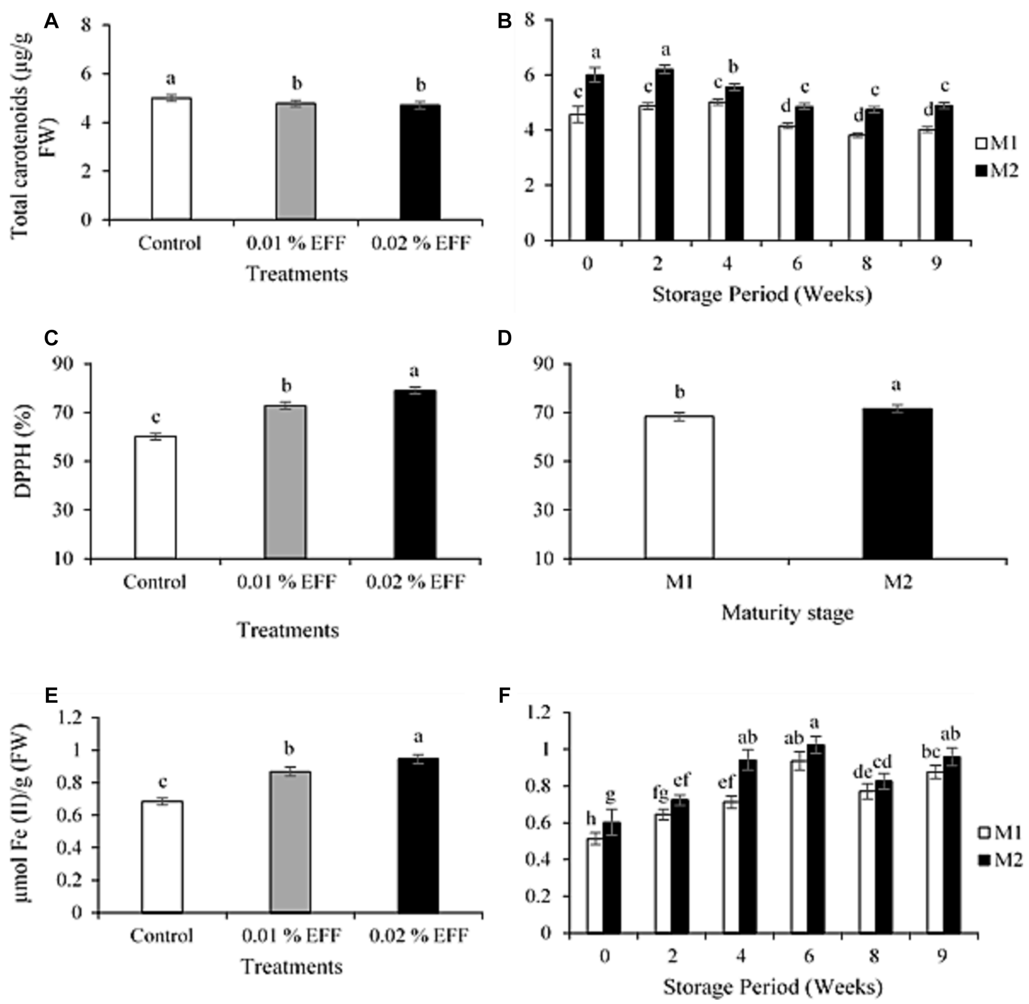

FIGURE 2
Effect of treatments **(A)** and the effect of interaction between maturity stage and storage time **(B)** on the total carotenoid content of kiwifruit. Effect of treatments (C) and maturity stage **(D)** on DPPH scavenging activity of kiwifruit. Effect of treatments **(E)** and the effect of interaction between maturity stage and storage time **(F)** on FRAP of kiwi fruit. Values are the means ± SE (*n* = 3).

in the degradation of carotenoids, resulting in a decrease in carotenoid content (Xia et al., 2020).

Antioxidant capacity

The interaction between treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on DPPH. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on DPPH. Treatments had a significant effect ($p < 0.001$) on DPPH scavenging activity (Figure 2C). Treated fruit had higher DPPH scavenging activity relative to the control. The maturity stage had a significant ($p < 0.01$) effect on DPPH scavenging activity (Figure 2D). Kiwifruit harvested at M2 exhibited greater DPPH scavenging activity compared to kiwifruit harvested at M1. The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on the Ferric Reducing Antioxidant Power (FRAP). The interaction between treatment and storage time had no significant effect ($p > 0.05$) on FRAP. Treatments had a significant effect ($p < 0.001$) on FRAP (Figure 2E). The control, 0.01 and 0.02% EFF treatment level had 0.68, 0.87 and 0.95 µmol Fe (II) g^{-1} FW, respectively. The interaction between maturity stage and storage time had a significant effect ($p < 0.05$) on the FRAP (Figure 2F), with M2 registering significantly greater FRAP relative to M1. The FRAP significantly increased and decreased during cold storage, followed by an increase during shelf life. The findings are comparable with those attained by Jincy et al. (2017) and Öz et al. (2023), where hexanal treated mango and persimmon fruit had higher antioxidant capacity.

Bioactive compounds such as ascorbic acid, polyphenols and flavonoids in plants have a significant influence on the antioxidant capacity (He et al., 2019). Thus, DPPH and FRAP assays were performed to assess the antioxidant capacity of these bioactive compounds. EFF treatment application substantially ($p < 0.05$) increased the antioxidant capacities, which is in accordance with results attained by Jincy et al. (2017), for EFF-treated mango fruit. Therefore, the enhanced antioxidant capacity exhibited by EFF-treated kiwifruit may be owed to a higher content of bioactive compounds in treated fruit (Figure 1). Furthermore, the findings of this study show that the maturity stage significantly influenced the antioxidant capacity of kiwifruit (Figures 2D,F). Kiwifruit harvested at M2 exhibited greater antioxidant capacity than kiwifruit harvested at M1. Considering that bioactive compounds influence antioxidant capacity, these findings indicate that the higher antioxidant capacities of kiwifruit harvested at M2 is attributed to their higher concentration of bioactive compounds (Figure 1). These results are in alignment with those of Lim and Eom (2018), who demonstrated that kiwifruit harvested at later maturity stages exhibited a greater concentration of bioactive compounds. The findings obtained in the current study, indicate that EFF treatment effectively optimized the bioactive compounds and antioxidant capacity in kiwifruit at both maturity stages.

Phenylalanine ammonia-lyase and tyrosine ammonia-lyase

The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on PAL activity. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on PAL. Treatments significantly ($p < 0.001$) influenced the PAL enzyme activity (Figure 3A). The control, 0.01 and 0.02% EFF treatment level registered 1.11, 1.32 and 1.41 U g^{-1} FW, respectively. The maturity stage had a significant ($p < 0.001$) effect on PAL activity (Figure 3B), with kiwifruit harvested at M2 (1.46 U g^{-1} FW) exhibiting greater PAL activity compared to kiwifruit harvested at M1 (1.08 U g^{-1} FW). The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on TAL. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on TAL. The treatments significantly ($p < 0.001$) influenced TAL activity (Figure 3C), with treated fruit exhibiting higher TAL enzyme activity relative to the control. A significant difference was not observed among treated fruit. The interaction between maturity stage and storage period had a significant effect ($p < 0.001$) on the TAL activity (Figure 3D).

Results obtained from this study are in accordance with those reported by Bai et al. (2014) and Dhakshinamoorthy et al. (2020), who illustrated that hexanal treatment enhanced the PAL activity of oriental sweet melon and banana fruit, respectively. To the best of our ability, we have not found research studies reporting on the effect of hexanal or EFF on TAL enzyme activity. The phenylpropanoid biosynthesis pathway is a metabolic pathway in plants that utilities phenylalanine and in some instances tyrosine to synthesize secondary metabolites (de Barros Rates and Cesarino, 2023). This pathway is governed by the enzymatic action of PAL, which is involved in the synthesis of phenolic compounds such as phenolic acids and flavonoids (Barros et al., 2015; Bian et al., 2020). Similar to PAL, TAL is an enzyme in the phenylpropanoid biosynthesis pathway involved in the conversion of tyrosine to *p*-coumarate, and it also facilitates the production of secondary metabolites (Yadav et al., 2020).

The findings in this study suggest that EFF influenced the phenylpropanoid biosynthesis pathway by enhancing PAL and TAL enzyme activities in treated kiwifruit. Considering that PAL and TAL participate in the synthesis of phenolic compounds, the higher TPC and TFC (Figures 1A,C) in EFF-treated kiwifruit, may be attributed to the ability of EFF to enhance PAL and TAL enzyme activities. Previous research studies have shown that hexanal upregulates the expression of genes that encode for PAL enzyme activity (Padmanabhan et al., 2020; Hyun et al., 2022). Thus, we hypothesize that the enhanced enzyme activity of PAL in EFF-treated kiwifruit may be due to the upregulation of PAL genes in response to EFF treatment. However, further research investigating the response of gene expression to EFF-treated kiwifruit is warranted to strengthen this argument.

Ascorbate oxidase

The interaction between treatment, maturity stage and storage time had a significant ($p < 0.001$) effect on AO enzyme activity (Figures 3E,F). The AO enzyme activity decreased at both maturity stages during storage. However, storing the kiwifruit at 20°C after cold storage, led to an increase in AO activity. Kiwifruit harvested at M1 had higher AO activity (control = 9.93 U g^{-1} FW, 0.01% EFF = 6.11 U g^{-1} FW, 0.02% EFF = 4.21 U g^{-1} FW) in comparison to kiwifruit harvested at M2 (control = 9.69 U g^{-1} FW, 0.01% EFF = 4.50 U g^{-1} FW, 0.02% EFF = 3.91 U g^{-1} FW) at week nine of storage. The treatments significantly ($p < 0.001$) influenced the AO activity, with the treated fruit exhibiting significantly lower AO enzyme activity compared to the control. Considering that AO is involved in the degradation of ascorbic acid (Chatzopoulou et al., 2020), the efficacy of EFF to optimize retention of ascorbic acid (Figure 1E) may be attributed to its capacity to suppress the enzymatic activity of AO. A qRT-PCR analysis conducted by El Kayal et al. (2017) demonstrated that EFF treatment application downregulated the gene expression of AO in strawberry fruit. Thus, we hypothesize that the mechanism in which EFF suppresses the enzymatic activity of AO is due to its ability to downregulate the gene expression of AO, which in turn, leads to a reduction in the oxidation of ascorbic acid.

Polyphenol oxidase activity

The interaction among treatment, maturity stage and storage time had no significant ($p > 0.05$) effect on PPO enzyme activity. The interaction between treatment and storage time had no significant effect ($p > 0.05$) on PPO. The treatments significantly ($p < 0.001$) influenced the PPO activity (Figure 3G). The control had the highest PPO activity, followed by the 0.01 and 0.02% treatment level. The interaction between maturity stage and storage period had a significant effect ($p < 0.01$) on the PPO enzyme activity (Figure 3H). Kiwifruit harvested at M2 exhibited significantly higher PPO activity than those harvested at M1. PPO was 0.13 and 0.16 U g^{-1} FW for M1 and M2 at week 9 of storage, respectively.

The findings of the present study are in accordance with those reported by Kaur et al. (2019) and Sharma et al. (2023), who demonstrated that EFF suppressed the PPO enzyme activity in oriental sweet melons, grape berries and jujube fruit, respectively. PPO is a terminal oxidase that utilizes polyphenols as substrates, which leads to internal browning of fruit. Furthermore, the enzymatic activity of PPO can be inhibited by aliphatic alcohols (Valero et al., 1990). Past research has demonstrated that hexanal in fruit tissues is converted to hexanol which is an aliphatic alcohol (Sharma et al., 2008). The lower PPO enzyme activity exhibited in treated fruit may be attributed to the conversion of hexanal to hexanol in kiwifruit tissues after EFF treatment application. To the best of our abilities,

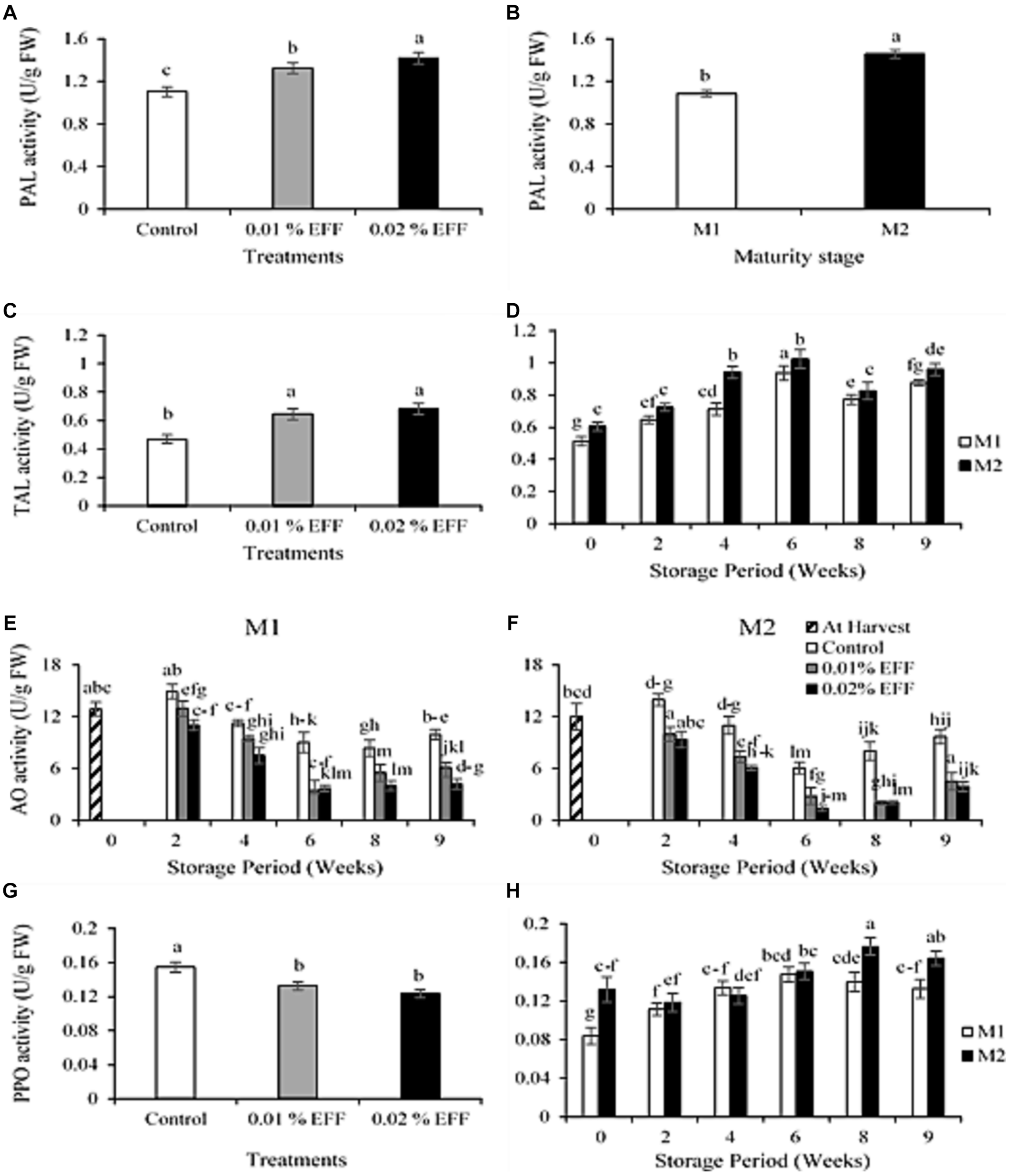

FIGURE 3
Effect of treatments **(A)** and maturity stage **(B)** on PAL activity of kiwifruit. Effect of treatments **(C)** and the effect of interaction between maturity stage and storage time **(D)** on TAL activity of kiwifruit. The effect of interaction among treatments, maturity stage and storage time on AO activity of kiwifruit harvested at M1 **(E)** and M2 **(F)**. Effect of treatments **(G)** and the effect of interaction between maturity stage and storage time **(H)** on PPO activity of kiwi fruit. Values are the means ± SE ($n = 3$).

we have not found studies investigating the capability of hexanol to inhibit PPO enzyme activity. Therefore, further research must be conducted to examine if the hexanol volatile compound has an inhibitory effect on the enzymatic activity of PPO.

Pearson correlation coefficient

A correlation analysis was done to examine the relationships between bioactive compounds on antioxidant capacity and the enzymes (Figure 4). The DPPH scavenging activity exhibited a significant and positive correlation with TPC ($r = 0.67$; $p < 0.001$), TFC ($r = 0.62$; $p < 0.001$) and AA ($r = 0.38$; $p < 0.001$). FRAP showed a significant and positive correlation with TPC ($r = 0.81$; $p < 0.001$), TFC ($r = 0.67$; $p < 0.001$) and AA ($r = 0.50$; $p < 0.001$). The correlation between PAL activity, TPC and TFC was 0.78 ($p < 0.001$) and 0.76 ($p < 0.001$), respectively. This indicates that TPC and TFC is strongly correlated with PAL enzyme activity. TAL activity exhibited a strong positive correlation with TPC ($r = 0.71$; $p < 0.001$) and a weak correlation with TFC ($r = 0.39$; $p < 0.001$). AA content and AO enzyme activity exhibited a significant but moderately negative correlation ($r = -0.44$; $p < 0.001$).

The results from the correlation analysis show that bioactive compounds have a direct influence on the antioxidant capacity of

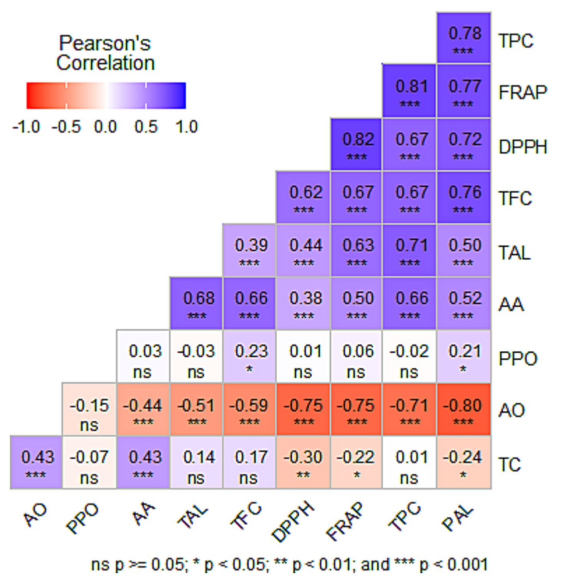

FIGURE 4
Pearson correlation matrix between total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid (AA), total carotenoids (TC), DPPH, Ferric-Reducing Antioxidant Power (FRAP), phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), ascorbate oxidase (AO), and polyphenol oxidase (PPO). The correlation coefficients are proportional to the color intensity. Positive correlation is displayed in blue and negative in red color. n.s. = not significant.

kiwifruit, which is in alignment with findings reported by He et al. (2019). Furthermore, the correlation analysis indicates that the accumulation of phenolic compounds is associated with the enzymatic activity of PAL and TAL. These findings are corroborated by those reported by Khan et al. (2003). Furthermore, the inverse relationship between AA and AO suggests that AO has an influence in the degradation of AA (Jiang et al., 2018). These findings indicate that the bioactive compounds in kiwifruit can be optimized by enhancing the activities of enzymes involved in the synthesis of phenolic compounds and suppressing the enzyme (AO) involved in the degradation of AA. Therefore, the efficacy of EFF to optimize the bioactive compounds in kiwifruit, could be attributed to its capacity to enhance and suppress the activity of enzymes involved in the synthesis and oxidation of these bioactive compounds, which in turn improve kiwifruit quality and storability.

Conclusion

The findings from the present study showed that EFF effectively optimized the antioxidant capacity in kiwifruit by regulating the action of enzymes involved in both the synthesis and oxidation of bioactive compounds. Therefore, it can be concluded that EFF has the potential to optimize the functional quality of South African grown gold kiwifruit, harvested at the mid and late maturity stage. The present study primarily examined the effect of EFF on the activity of enzymes involved in both the synthesis and oxidation of bioactive compounds. However, transcriptome analysis is needed in order to gain an in-depth understanding of the underlying mechanism on how EFF influences these enzymes. Therefore, further research using RNA sequencing is warranted to provide a comprehensive understanding of the mechanism in which EFF optimizes the synthesis of bioactive compounds. Furthermore, assessing the inhibitory effects of the hexanol volatile compound against PPO activity, may elucidate how hexanal application suppresses the action of this enzyme.

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

SM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. LM: Conceptualization, Funding acquisition, Supervision, Validation, Visualization, Writing – review & editing. ST: Conceptualization, Data curation, Supervision, Validation, Writing – review & editing. AM: Conceptualization, Supervision, Writing – review & editing, Project administration.

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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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Online monitoring system of material moisture content based on the Kalman filter fusion algorithm in air-impingement dryer

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A Kalman filter fusion algorithm was proposed, and an online monitoring system was developed for real-time monitoring of the moisture content of materials in an air-impingement dryer. The Kalman filter algorithm was used to estimate the optimal state of the original detection values of the weighting sensor and air velocity sensor. A backpropagation (BP) neural network fusion model was established, where the weight detection value, elastic substrate temperature, air velocity, and impingement distance were considered inputs and the real weight of the material was the output. The optimal topology of the BP neural network was selected, and the initial weights and thresholds of the BP neural network were optimized using a genetic algorithm. The coefficient of determination (R^2) and root mean square error (RMSE) of the optimized BP neural network fusion model were 0.9995 and 4.9, respectively. The Kalman filter fusion algorithm, which can realize online monitoring of moisture content, was established using the Kalman filter algorithm and fusion model. Moreover, an online monitoring system for material moisture content was developed, validation experiments were carried out, and the R^2 and RMSE of the nine sets of validation experiments were 0.9963 and 0.78, respectively. The monitoring system satisfied the requirements of material moisture content detection accuracy in the drying process. The developed monitoring system is greatly important for improving the automation level of the drying equipment for fruits and vegetables. The proposed Kalman filter fusion algorithm also provides a reference for other multifactor fusion detection.

KEYWORDS

online monitoring, moisture content, Kalman filter, neural network, fusion model

1 Introduction

Food security and storage have always been one of critical challenges worldwide, especially for fruits and vegetables, which have severe postharvest losses (Zartha Sossa et al., 2021). At present, one of the best ways to solve this problem is drying. By reducing the moisture content to a safe level, drying can significantly minimize deterioration due to microbial conditions, extend the shelf life of products, reduce the cost of packaging, transportation, and storage, and effectively reduce postharvest losses (Xiao and Mujumdar, 2020). Air-impingement drying technology realizes drying through the impact and heating of hot air on the material; it is crucial for post-harvest drying treatment of agricultural products. Air-impingement drying technology is characterized by a high drying rate and high heat transfer coefficient (Yang et al., 2023) and has been widely used in the drying process of kumquat (Tan et al., 2022), tomato (Tan et al., 2021), kiwifruit (Li et al., 2023), apple (Peng et al., 2019), shiitake mushroom (Liu et al., 2020), and other agricultural products.

Moisture content is a critical parameter in air-impingement drying and other drying processes, and its online monitoring plays a vital role in improving the drying automation level, optimizing the drying process, and ensuring the continuity of drying. Existing online moisture content detection methods include the model prediction method (Dalvi-Isfahan, 2020), dielectric properties method (Celik et al., 2022), hyperspectral imaging method (Cho et al., 2020), nuclear magnetic resonance method (Li et al., 2021), and weighing method (Pongsuttiyakorn et al., 2019). The model prediction and dielectric characterization methods only apply to the moisture content detection of the same material in a fixed drying condition and require improved versatility. Hyperspectral imaging and nuclear magnetic resonance methods cannot be applied to agricultural drying products with low added value due to their high cost and complex operation steps. The weighing method is not limited by the type of material and drying condition and is more versatile. At the same time, it is widely used for online monitoring of moisture content in the drying process of fruits and vegetables due to its low cost and simple operation.

The weighing method utilizes the oven method to obtain the initial moisture content of a batch of materials (Wang et al., 2022). Under the premise of defaulting to the same batch of materials with the same initial moisture content, based on the principle of a constant mass of dry matter in the drying process, the weighing method converts the material's weight in the drying process into moisture content. The key to realizing accurate detection of moisture content of materials in the drying process by weighing method is the accurate measurement of real-time weight of materials using a weighting sensor. The uncertainty of the drying environment caused substantial difficulties in realizing the accurate measurement of material weight. The impact of the airflow on the weighing tray during the drying process directly and remarkably affects the detection results of the weighting sensor (Xie et al., 2018). Moreover, in different drying processes with different set values of airflow velocity, the influence of airflow on the moisture content detection results is not fixed. The weighting sensors used for online monitoring of moisture content are mostly resistance strain pressure sensors. Variation in the temperature of the sensor's elastic substrate can cause many errors in the detection results of the weighing sensor given some unique properties of this sensor (Xue et al., 2018). The variation of temperature in the existing drying process of fruits and vegetables is roughly in the range of 40°C–70°C. The effect of drying temperature on the material weight detection results becomes nonlinear and complex to correct due to the considerable temperature difference. In addition, airflow disturbances and equipment vibrations cause fluctuations in the detection value of the weighting sensor with considerable noise.

To address these problems, Ju et al. (2023), Yang et al. (2023), and Wang et al., 2014 stopped the equipment operation while performing the material moisture content detection. This method effectively avoided the influence of airflow and equipment vibration on material weight detection. The moisture content of the material in the drying process must be detected more frequently with the development of the drying process and the improvement of drying automation. In this context, the equipment-stop detection program seriously damages the continuity of the material drying process, and ensuring the quality of dry products becomes challenging. To address the nonlinear effect of drying temperature on the measurement results of the weighting sensor, Reyer et al. (2022) installed the weighting sensor outside the drying chamber, thereby fundamentally solving the problem. However, this solution destroyed the drying chamber structure, affected the sealing of the drying chamber, and increased the difficulty of temperature and humidity control in the drying chamber.

The air-impingement drying process can be optimized by varying the distance between the air nozzle and the material, i.e., the impingement distance. Pre-experimentation found that the impingement distance significantly affected the weighting sensor's detection value. In summary, the real-time moisture content detection of the material in the drying process is affected by the weight detection value, the elastic substrate temperature, the airflow velocity, and the impingement distance. A complex nonlinear relationship exists between the material moisture content and the four factors. Machine learning can model the complex nonlinear relationship between the variables. Backpropagation (BP) neural network, a type of machine learning, has been widely used in the fields of drying process modeling (Liu et al., 2020), trajectory prediction (Zheng et al., 2023), fault diagnosis (Wang et al., 2023), and residual life prediction (Ji et al., 2023) because of its powerful nonlinear mapping ability. The BP neural network can be used to build a nonlinear model with weight detection value, elastic substrate temperature, air velocity, and impingement distance as inputs and the real weight of the material as output, resulting in an accurate prediction of the moisture content of the material during the drying process.

The weight of the material, the temperature of the elastic substrate, and the air velocity can be detected sequentially by weighting, temperature, and air velocity sensors. Many fluctuations and noise are observed in the detection values of the three sensors, especially the weighting and air velocity sensors, due to the airflow disturbance, equipment vibration, and the sensors' limitations. Therefore, the raw signals obtained from the sensors should be filtered prior to building the moisture content prediction model. Kalman filter is an efficient autoregressive filter that can estimate the state of the dynamic system in the information, where many uncertainty situations exist and can make an optimal estimation of the detected values of the three sensors mentioned above (Li et al., 2022).

Aiming at these problems, the Kalman filter fusion algorithm was proposed. The Kalman filter algorithm was used to make an optimal estimation of the measured values of the weight sensor, air velocity sensor, and temperature sensor at the same moment. Then, the BP neural network was used to establish a fusion model among the optimal estimation of the three sensors, the impingement distance, and the real weight of the material. Combined with the initial moisture content of the material, the online detection model of moisture content of the material was established, and the Kalman filter fusion algorithm was completed. The online monitoring system of material moisture content was built using this algorithm.

In summary, this study aims (1) to build an information acquisition system of material moisture content in the air-impingement dryer and to obtain the training data used to establish the Kalman filter fusion algorithm; (2) to develop an optimal state estimation of the original monitoring data of the three types of sensors by using the Kalman filter algorithm; (3) to train the BP neural network, and to establish a fusion model with the weight detection value, elastic substrate temperature, air velocity, impingement distance as inputs, and the real weight of the material as outputs; (4) to build an online monitoring system for moisture content and complete the validation experiments of online moisture content detection. This study is expected to realize the online monitoring of the moisture content of materials in air-impingement drying for fruits and vegetables.

2 Principles and methods

2.1 Operating principle of air-impingement dryer

The centrifugal fan realized the internal air circulation in the inner and outer chambers. The air passing through the nozzles was squeezed into a high-pressure stream, impacting the material on the trays. The infrared tube installed in the upper part of the inner chamber heated the materials on the tray. The material was dried under the double effect of airflow impact and heating. The wet air was discharged through the moisture drain valve in the outer chamber. The operation principle of the air-impingement dryer is shown in Figure 1.

A temperature sensor detected the temperature of the hot air at the nozzle. The closed-loop adjustment of the hot air temperature was realized by adjusting the power of the infrared tube. The detection result of the thermosensitive air velocity sensor was greatly affected by temperature. Thus, accurate detection of the airflow velocity in the drying inner chamber is difficult at different drying temperatures. Consequently, the centrifugal fan realized the open-loop control of wind speed by adjusting the frequency of the inverter (Yang et al., 2023). The open-loop control of the centrifugal fan also obtained an unstable airflow in the inner chamber.

In the air-impingement drying process, in addition to the drying temperature and air velocity, the tray position was an essential parameter for process optimization (Chang et al., 2022). The positions of the tray in the material rack, the temperature of the material surface, and the air velocity were different, as well as the final quality of the dry products obtained.

2.2 Principle of material moisture content detection based on the weighing method

The material moisture content detection prerequisite, based on the weighing method, was obtaining the material's initial moisture content. The dry matter mass of the material m_d was obtained after the material was continuously dried in a hot air dryer at a drying temperature of 105°C for 24 h (Zahoor et al., 2022). The initial moisture content of the material (wet basis) was calculated from Equation 1 (Liu et al., 2021).

$$W_0 = \frac{m_0 - m_d}{m_0} \times 100\% \quad (1)$$

where W_0 was the initial moisture content of the material, %; m_0 was the initial weight of the material, g; m_d was the dry matter mass of the material, g.

Material moisture content detection based on the weighing method defaulted to the same batch of material with the same initial moisture content. It converted the real-time weight of the material in the drying process to the moisture content of the material. The formula for calculating the real-time moisture content (wet basis) of the material is as follows:

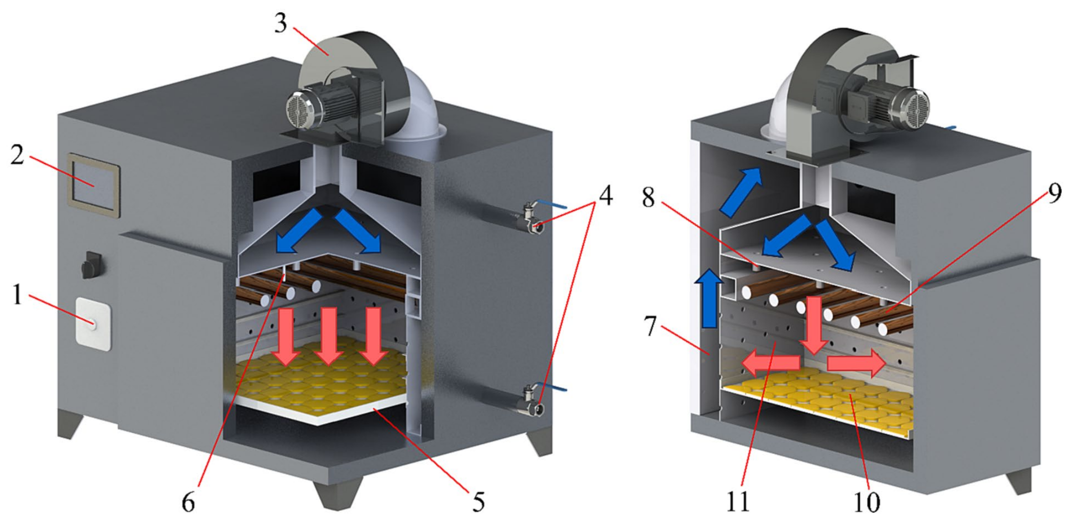

FIGURE 1
Operation principle diagram of air-impingement dryer. (1) Air velocity regulating switch; (2) touch panel; (3) centrifugal fan; (4) moisture drain valve; (5) tray; (6) temperature sensor; (7) outer chamber; (8) air nozzle; (9) infrared tube; (10) material; (11) inner chamber.

$$W_t = \frac{m_t - m_0\left(1 - W_0\right)}{m_t} \times 100\% \quad (2)$$

where W_t is the wet base moisture content of the material at time t, %; m_t is the weight of the material at time t, g.

The above principle indicates that the accurate detection of material weight by the weighting sensor is the core to ensure the accuracy of moisture content detection based on the weighing method. According to the previous experiment and theoretical analysis, air velocity, weighting sensor elastic substrate temperature, impingement distance, and equipment vibration affect the detection of the weighing sensor. Among them, equipment vibration only caused fluctuation and noise to the measurement value of the weighting sensor without direct influence. Airflow disturbances also exacerbated the instability of the weighting sensor measurements.

2.3 Information acquisition of material moisture content

The information acquisition system of material moisture content was built to obtain the training data for the Kalman filter fusion algorithm. The information acquisition system utilized the weighting sensor, air velocity sensor, and temperature sensor to collect the raw data of material weight detection value, elastic substrate temperature, and air velocity, respectively. The flow of material moisture content information acquisition is shown in Figure 2.

2.3.1 Information acquisition system of material moisture content

The weighting sensor was installed at the bottom of the material rack and was used to detect the real-time weight of the material. The weighting sensor was a resistance strain pressure sensor (HYPX017, Hengyuan Sensor Technology Co., Ltd., Bengbu, China) ranging from 0–3 Kg. The temperature of the outer chamber was significantly lower than that of the inner chamber due to the lack of heating from the infrared tube. The air velocity sensor was installed in the outer chamber to avoid the influence of temperature on the measured value of the air velocity sensor. The thermosensitive air velocity sensor (WM4200, Chaozhi Reed Technology Co., Ltd., Changchun, China) has a range of 0–20 m/s. A temperature sensor was used to detect the temperature of the elastic substrate of the weighting sensor. A thermally conductive silicone was used to bond the temperature sensor to the elastic substrate. The temperature sensor was a thermocouple-type (PT100, Songguide Heating Sensor Co., Ltd., Shanghai, China) with a range of −45°C–125°C. All three sensors communicated with the host computer by RS485 module. The upper computer was a Legion Y7000P computer from Lenovo, which was responsible for collecting and storing material moisture content information.

2.3.2 Experimental design of information acquisition of material moisture content

The information acquisition experiments of material moisture content were carried out under different drying process parameters to ensure the accuracy of the Kalman filter fusion algorithm. The range of drying process parameters was initially defined, and that of drying temperature for fruits and vegetables was generally 40°C–70°C. An extremely temperature reduces the drying rate and significantly increases the total energy consumption of the equipment. An extremely high temperature causes the material to produce scorching, browning, and other phenomena, significantly reducing the quality of dry products (Espinoza-Espinoza et al., 2023). The air velocity in the outer chamber was 16 m/s when the centrifugal fan operated at the maximum output wind speed. The minimum air velocity was set to 4 m/s to prevent the phenomenon of low drying rate caused by extremely low air velocity. The structure of the material rack determined the impingement distance. Three layers of material racks are available, and the distance of each layer from the nozzle was 80,

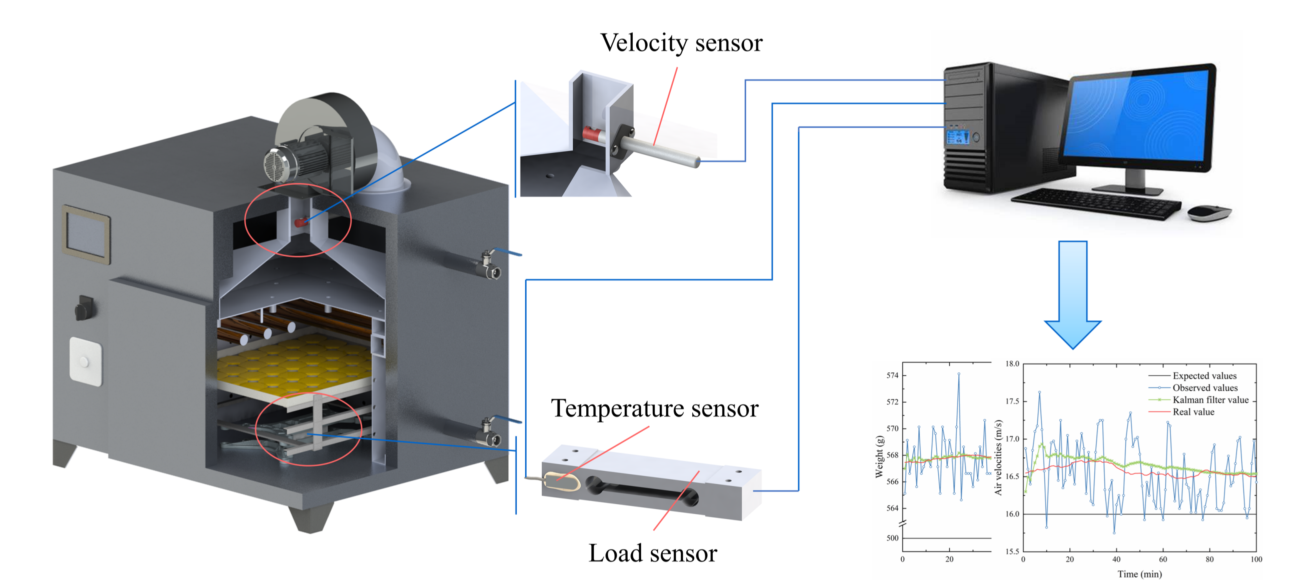

FIGURE 2
Flow chart of information acquisition of material moisture content.

120, and 160 mm. In summary, the information acquisition experiments of material moisture content were carried out at different drying temperatures (40°C, 50°C, 60°C, and 70°C), different air velocities (4, 8, 12, and 16 m/s), and different impingement distances (80, 120, and 160 mm), totaling 48 groups of experiments (4 × 4 × 3). In each group of experiments, the information acquisition period of material moisture content was 1 min.

Fresh cantaloupe (variety Xizhou Honey No. 17), free from pests, diseases, and damage, was selected as the experimental material. After being peeled and deseeded, the cantaloupe was cut into 30 × 50 × 7 mm slices for drying experiments. The average initial moisture content of fresh cantaloupes was 90.16 ± 1.02% (wet basis).

2.4 Kalman filter processing of sensor data

Equipment vibration and airflow disturbance affected the weighting sensor detection signal, and its noise was the most serious. The air velocity sensor was affected by the uncertainty of the dry environment; coupled with the limitations of the sensor, its detection signal had considerable noise and fluctuations. Noise in the detection signals of the two sensors was difficult to avoid. The original detection signals of the two sensors should be filtered prior to data fusion.

The temperature of the elastic substrate of the weighting sensor was less affected by the radiation of the infrared heating tube given that the weighting sensor was mounted on the bottom of the pallet. Changes in the elastomeric substrate temperature were caused by heat conduction with the air in the inner chamber, achieving a relatively stable elastic substrate temperature without significant fluctuations. Preliminary experiments revealed that the detected value of the elastic substrate temperature was 70 ± 0.5°C during the constant temperature drying process at 70°C. Therefore, in this study, only the detection data of the weight sensor and air velocity sensor were subjected to Kalman filtering.

Kalman filtering algorithm is developed for optimal state estimation of a linear system using the equation of the system state and the observed data of the system's current state (You et al., 2022). The optimal estimation can also be considered a filtering process because the observation data include a large amount of noise and disturbance. The Kalman filtering algorithm can reduce the random measurement errors caused by the sensor's limitations during the sensor data acquisition process and the noise pollution caused by uncertain environmental factors. Kalman filtering carries out the next operation using the results of the previous operation without storing the data; its operation cycle is short and is suitable for the real-time state estimation process (Yang et al., 2021). Kalman filtering can update and process the data collected in the field in real-time. It can be used to estimate state variables that cannot be accurately measured by the system.

The flow of the Kalman filter algorithm can be divided into two phases: the prediction update phase and the observation update phase. In the prediction update phase, the current state was predicted using the previous moment state estimate. In the observation update phase, the observed value of the current state was used to correct the predicted value in the prediction update phase to obtain a relatively accurate state estimate of the current moment.

The discrete equation of state for the linear system was initially established, as follows:

$$x(k) = Ax(k-1) + w(k-1) \tag{3}$$

$$y(k) = Hx(k) + v(k) \tag{4}$$

where $x(k)$ is the state vector of the system; A is the state transfer matrix; $y(k)$ is the observation value; H is the observation matrix; $w(k)$ and $v(k)$ are the process noise and observation noise, respectively.

In the prediction update phase, the system state at time k was predicted based on the system state at time $k-1$, as follows:

$$x(k|k-1) = Ax(k-1) \tag{5}$$

where: $x(k|k-1)$ is the state prediction result at time k; $x(k-1)$ is the optimal state estimate at time $k-1$.

The covariance of the system at time k should also be forecasted during the prediction update phase of the system, as follows:

$$P(k|k-1) = AP(k-1)A^T + Q \tag{6}$$

where $P(k|k-1)$ is the prediction of the covariance of the system at time k; $P(k-1)$ is the covariance of the system at time $k-1$, and Q is the covariance of the system process noise.

In the observation update stage, the Kalman gain $K_g(k)$ at time k was initially computed using the prediction covariance at time k and the covariance R of the observation error $v(k)$, as follows:

$$K_g(k) = P(k|k-1)H^T\left(HP(k|k-1)H^T + R\right)^{-1} \tag{7}$$

Finally, the optimal state estimate $x(k)$ at time k was obtained, as follows:

$$x(k) = x(k|k-1) + K_g(k)\left(y(k) - Hx(k|k-1)\right) \tag{8}$$

Corrections were made to $x(k)$ to obtain the real value at moment k:

$$\hat{x}(k) = Ax(k) + w(k) \tag{9}$$

The covariance of the system at time k was also updated for the next Kalman filter run, as follows:

$$P(k) = \left(I - K_g(k)H\right)P(k|k-1) \tag{10}$$

where I is the unit matrix. When the system entered the time $k+1$, $P(k)$ is equal to $P(k-1)$, and the Kalman filter continued running until the end of the process.

2.5 Fusion modeling

Kalman filtering of sensor detection data provided statistically accurate and valid data for data fusion. This study used a BP neural network for data fusion of weight detection value, elastic substrate temperature, air velocity, and impingement distance. Moreover, the Kalman filter fusion algorithm was established. The flowchart of the Kalman filter fusion algorithm is shown in Figure 3. Furthermore, a fusion model based on partial least squares regression (PLSR) and support vector machine (SVM) was developed and compared with the BP neural network fusion model, and the effectiveness of the BP neural network fusion model was further verified.

2.5.1 BP neural networks

BP neural network, one of the most widely used neural network models, can learn and store many mapping relationships between inputs and outputs (Liu et al., 2020). In this study, the BP neural network was used to establish a fusion model, where the actual weight of the material is the output, and to accurately predict the moisture content of the material. The topology of the BP neural network model consisted of three layers of neurons and two activation functions between the three layers of neurons, as shown in Figure 4. The input layer had four neurons, representing the weight detection value, elastic substrate temperature, airflow velocity, and impingement distance. The output layer had only one neuron, representing the actual weight of the material. The number of neurons in the hidden layer must be obtained by trial and error. The activation function between the input and hidden layers is usually nonlinear, including the logsig and transig functions. The transfer function between the hidden and output layers is usually linear (Pureline) (Yang et al., 2023). The optimal topology of the network, i.e., the number of neurons in the hidden layer and the activation function between the input layer and the hidden layer, should be determined to improve the generalization ability of the model and reduce the training time of the network (Liu et al., 2020).

$$j < n - 1 \tag{11}$$

$$j < \sqrt{m + n} + a \tag{12}$$

$$j = \log_2 n \tag{13}$$

$$j = 2n + 1 \tag{14}$$

where n is the number of neurons in the input layer, m is the number of neurons in the output layer, j is the number of neurons in the hidden layer, and a is a constant from 0–10.

The number of neurons in the hidden layer ranged 2–12 based on the union of Equations 11–14 (Martin and Howard, 2002). The optimal topology of the neural network was determined based on the root mean square error (RMSE) and the coefficient of determination (R^2) of the model test set (Kalathingal et al., 2020). The R^2 and RMSE were calculated using Equations 15 and 16, respectively.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(\hat{y_i} - y_i\right)^2}{\sum_{i=1}^{n}\left(y_i - y_m\right)^2} \tag{15}$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}\left(\hat{y_i} - y_i\right)^2}{n}} \tag{16}$$

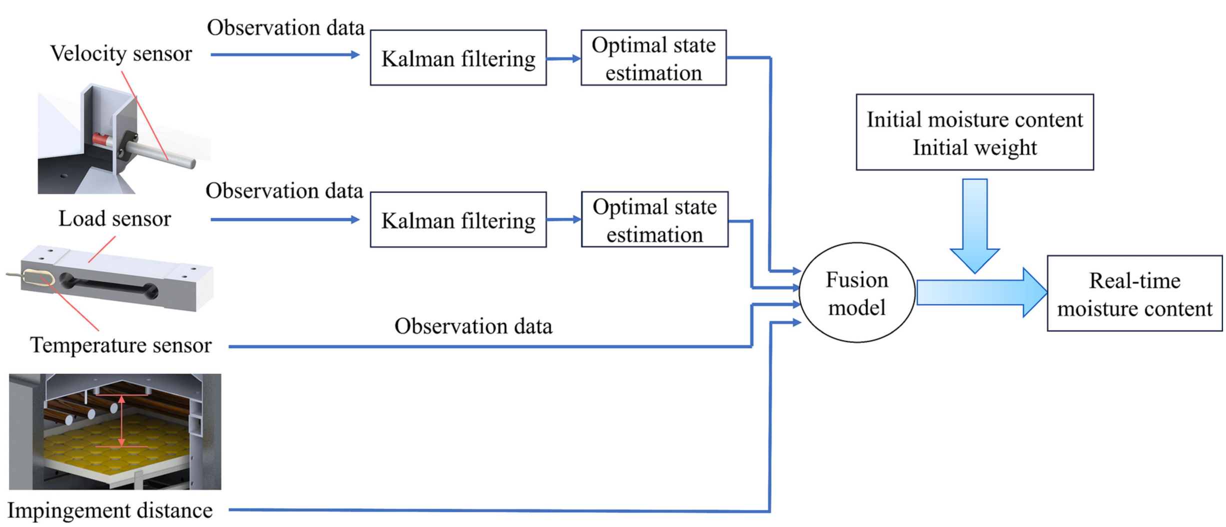

FIGURE 3
Flowchart of Kalman filter fusion algorithm.

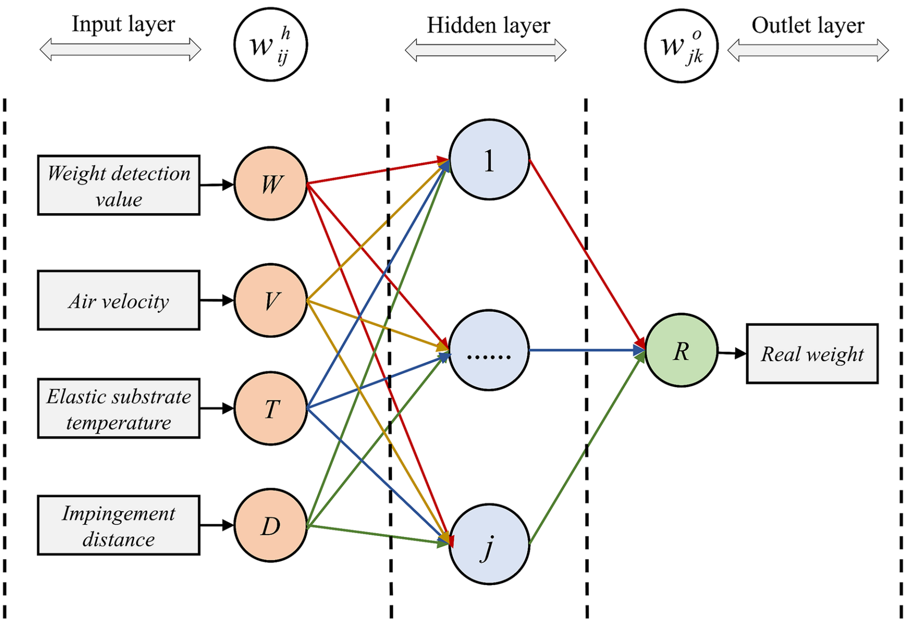

FIGURE 4
Topology of the BP neural network model.

Where n is the number of data groups in the set, y_i and $\hat{y}_i$ are the real value and predicted value of the i^{th} data groups, and y_m is the mean value of all the data groups.

Neural networks underwent network training after obtaining the optimal topology. The essence of neural network training was the optimization of weights and thresholds. The initial weights and thresholds of the neural network were randomly generated. However, the training results of the neural network were sensitive to the initial weights and thresholds. A genetic algorithm with global optimization characteristics was used to optimize the initial weights and thresholds of the neural network to ensure the accuracy and generalization ability of the model (Raj and Dash, 2020). The neural network was trained based on the optimized initial weights and thresholds.

Ten data groups were randomly selected from each set of information acquisition experiments of material moisture content, totaling 480 groups of data (48 × 10) for the training of the fusion model. Each group of data included four input variables and one output variable. A total of 480 groups of data were randomly sorted to ensure the reliability of the training results: 70% of the data were used as training data, 15% as validation data, and 15% as test data.

2.5.2 Partial least squares regression

The central idea of PLSR is to maximize the synergistic explanation of the relationship between the independent and dependent variables by finding one or more latent factors. These latent factors construct the model by linearly combining the independent variables, and their weight coefficients are determined by minimizing the covariance between the predicted and true values. PLSR has significant advantages in dealing with high-dimensional data and small samples and is an essential tool in data modeling and analysis (Camarrone and Van Hulle, 2019). PLSR has shown excellent predictive performance in several areas.

The input variable matrix and output variable matrix are decomposed:

$$X = DP^T + E \quad (17)$$

$$Y = UQ^T + F \quad (18)$$

Where $X \in R^{N \times M}$, is the input variable matrix, N is the number of samples, and M is the dimension of the input variable; D, U are the principal factor score matrices; P, Q are the loading matrices; E, F are the fitted residuals matrices; and $Y \in R^{N \times K}$, is the output variable matrix, and K is the dimension of the output variable.

The linear relationship between matrices D and U is:

$$U = DB \quad (19)$$

$$Y_{pre} = D_{pre}BQ \quad (20)$$

In Eq. (19) and Eq. (20), $B = \left(D^T D\right)^{-1} D^T U$ is the regression coefficient matrix. After that, the training set data is inputted, and its principal factor score matrix D_{pre} is obtained according to Eq. (17). Then the output variable matrix Y_{pre} is obtained according to Eq. (20). By projecting the regression coefficient matrix, the regression model of PLSR can be obtained.

2.5.3 Support vector machines

SVM is a robust learning algorithm for classification and regression tasks. Its main goal is to find a hyperplane that can classify data points into different fit data while maximizing the interval between the hyperplane and the nearest data points. SVM has excellent generalization performance, robustness, and applicability and is particularly suitable for dealing with high-dimensional, complex, or nonlinear datasets (Jain and Rastogi, 2022).

Define the training set $Q=\{(x_i,y_i)|i=1,2,\ldots,N\}$, where x_i is 4-dimensional as the input vector, y_i is the output vector, and N is the total number of samples. Taking the quadratic sum of the error e as the loss function, the SVM optimization problem can be expressed as (Ashtiani et al., 2020):

$$\min_{w,s,e} J(w,e)=\frac{1}{2}w^T w+\frac{1}{2}\lambda\sum_{i=1}^{N}e_i^2 \quad (21)$$

$$s.t.\, y_i = w^T \cdot \varphi(x_i)+s+e_i \quad (22)$$

Where w is the weight variable; λ is the regularization parameter, here 100; s is the threshold; e_i is the error value; $\varphi(x_i)$ is a nonlinear mapping in the kernel space. The original problem is transformed into the problem of finding the great value of the multiplier a_i according to the Lagrange multiplier method, and the following function is constructed:

$$L(w,s,e,\alpha)=J(w,e)-\sum_{i=1}^{N}\alpha_i\left(w^T\varphi(x_i)+s+e_i-y_i\right) \quad (23)$$

The regression function of SVM is:

$$f(x)=\sum_{i=1}^{N}\alpha_i K(x,x_i)+s \quad (24)$$

Where x is the input vector in the training set; K is the kernel function, and the radial basis function with a simple and stable structure is chosen as the kernel function, and its expression is:

$$K(x,x_i)=\exp\left(-\frac{\|x-x_i\|}{2\sigma^2}\right) \quad (25)$$

Where σ^2 is the width of the kernel function, according to Equation (24), to predict the real weight of the material.

2.6 System performance verification experiments

In the material moisture content online monitoring system based on the Kalman filter fusion algorithm, the upper computer was still Lenovo's Legion Y7000P computer. It was used to complete the collection of material moisture content information. In MATLAB software, the Kalman filter fusion algorithm was written, including of the following: the Kalman filter and BP neural network fusion model. The impingement distance should be input into the MATLAB software in advance given that the impingement distance may not be detected by the sensor. In addition, the material's initial weight and initial moisture content should be input into the MATLAB software to further calculate the real-time moisture content of the material when the real-time weight of the material was obtained by the Kalman filter fusion algorithm.

TABLE 1 Parameter settings for validation experiments.

Factor	Groups		
	1	2	3
Air velocity (m/s)	8.4	15.5	5.3
Impingement distance (mm)	60	140	100
Drying temperature (°C)	68	53	46

After completing the construction of the monitoring system, performance verification experiments were conducted, following the construction of the monitoring system. The drying experiments of jujube slices were carried out in three drying environments set at random to ensure the reliability of the results of the validation experiments. Three data groups were randomly collected in each drying experiment and input into the BP neural network fusion model to complete the moisture content detection. The time interval between each data acquisition group was greater than 15 min. After each data acquisition, the material tray was removed, and the material was weighed to determine the actual moisture content of the material according to the oven method in Section 2.2. The initial moisture content of the jujube pieces was 51.98% ± 0.50%.

The setup parameters of the three drying environments are shown in Table 1. The air velocity refers to the air velocity of the air duct in the outer chamber, measured by the air velocity sensor in Figure 2. The sectional dimension of the air duct is 60 mm × 70 mm. The impingement distance refers to the distance between the air nozzle and the material, as shown in Figure 3. Drying temperature refers to the air temperature in the inner chamber, measured by the temperature sensor in Figure 1.

3 Results and discussion

3.1 Results and analysis of Kalman filter processing

Figure 5 illustrates the estimated optimal state of the weight and air velocity sensors using the Kalman filtering algorithm. The expected value refers to the detected index in the ideal state. The observed value was the original value acquired by the sensor. The Kalman filter value was the optimal estimate of the system state made by the Kalman filter algorithm. The real value was not the real state of the measured metric but the ideal value without bias. The two data sets were obtained under the same set of experiments, with 100 sampling points, and the sampling interval at both experiments was 1 min.

Figure 5A shows the optimal state estimation results of the Kalman filtering algorithm for the original monitoring data of the weight sensor under the conditions of drying temperature of 70 °C, wind speed of 16 m/s, and constant load of 500 g. The fluctuation range of the observed values was 562.1–574.1 g, and the fluctuation amplitude was 12 g. The optimal state estimation results of the Kalman filtering algorithm were consistent with the real values, and the overall tendency was stable. Experimental results indicated that the Kalman filtering algorithm adequately suppressed the noise and fluctuations in the original monitoring data of the weight sensor. A significant

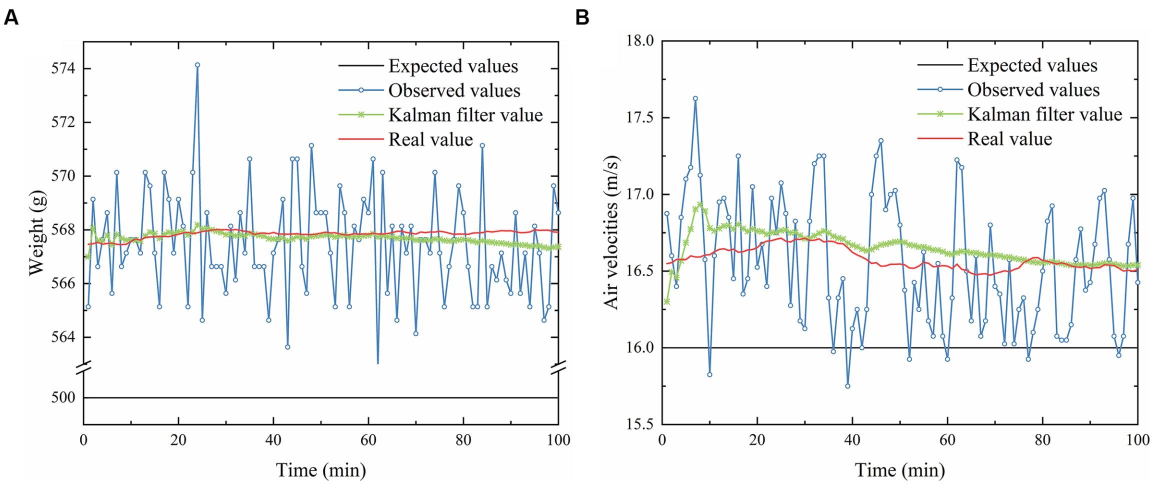

FIGURE 5
Optimal state estimation results of the Kalman filtering algorithm for the original monitoring data of the **(A)** weight sensor and **(B)** air velocity sensor.

error was found between the Kalman filtered value and the expected value, with a maximum error of 68.2 g. The detected value of the weighting sensor was affected by multiple factors other than the equipment vibration and the airflow disturbance, mainly the elastic substrate temperature and the air velocity. The experimental results also confirmed the need to establish a fusion model with multifactor inputs.

Figure 5B demonstrates the optimal estimation results of the Kalman filtering algorithm on the raw monitoring data of the air velocity sensor in the same set of experiments. The fluctuation range of the observed values was 15.75–17.63, and the fluctuation amplitude was 1.88 m/s. The Kalman filtered values of the air velocity sensor and the real values had evident fluctuations because the air velocity in the air-impingement dryer was open-loop control, and large fluctuations were observed in the wind speed. The air velocity of the centrifugal fan was controlled by adjusting the frequency of the inverter to realize the open-loop control, and the steady state error between the target value of air velocity and the output value was inevitable. Therefore, the Kalman-filtered and real values of the air velocity sensor had significant deviations from the expected value.

3.2 Pearson correlation analysis

Pearson correlation analysis of the four input variables and the detection error was conducted in this study to further quantitatively analyze the relationship between the weight detection value, elastic substrate temperature, airflow velocity, impingement distance, and the actual weight of the material. Detection error indicates the deviation between the detected value and the actual value of material weight. The results of the Pearson correlation analysis are shown in Figure 6.

The correlation between airflow velocity and detection error was as high as 0.811, suggesting that the leading cause was the airflow velocity. This result also proved the feasibility of using the air-stop detection scheme to detect the moisture content of the material in the existing studies. The positive correlation between air velocity and detection error was caused by the effect of the airflow on the material on the material tray from top to bottom. In addition, a large air velocity indicates large impact force of the airflow on the material tray and large detection.

The impingement distance negatively correlated with the detection error with a correlation coefficient of −0.357. The impingement distance represented the distance between the airflow nozzle and the tray. The larger distance indicates more dispersed airflow, smaller impact on the tray, and smaller detection error. The effect of the impingement distance on the detection error was highly dependent on the air velocity. When the air velocity is zero, the change in impingement distance does not affect the detection of material weight.

The temperature of the elastic substrate and the weight detection value were positively correlated with the detection error, with correlation coefficients of 0.226 and 0.329, respectively. In resistance strain pressure sensors, the pressure to be detected deforms the elastic substrate, which is transformed into a change in electrical resistance, thereby realizing the weight detection. The temperature significantly affected the resistance, exhibiting a positive correlation (Burnos and Rys, 2017). Therefore, when the temperature of the elastic substrate was high, the detection value of the weighting sensor was large, as well as the detection error.

The weight detection value and the detection error are correlated because the effect of temperature on the detection error was related to the load of the weighting sensor. The relationship between temperature and detection error was different at varied load intervals. The weight detection value had the most direct relationship with load; thus, the effect of temperature on the detection error resulted in the correlation between the weight detection value and the detection error. Wang et al. (2014) has linearly corrected the detection value of the weighing sensor in different subtemperature segments and subload segments.

3.3 Training results and analysis of BP neural network fusion models

After confirming the correlation between the weight detection value, elastic substrate temperature, airflow velocity, and impingement

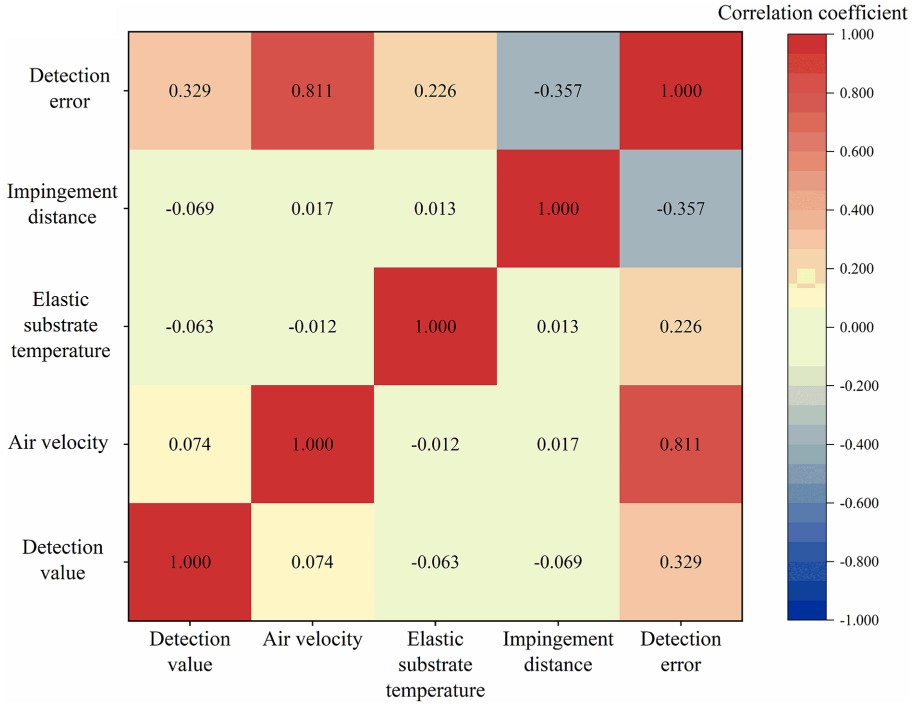

FIGURE 6
Results of Pearson correlation analysis.

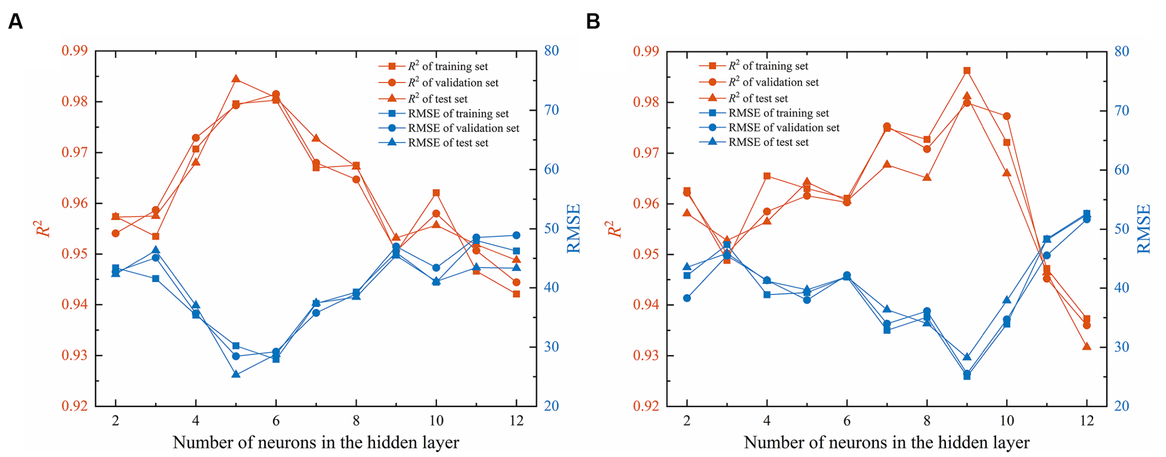

FIGURE 7
Prediction results for the test set of BP neural network fusion models. The fitting plot **(A)** and error plot **(B)** of the predicted and actual values of the test set.

distance on the true weight of the material, the four input variables were fused to establish a BP neural network fusion model with the actual weight of the material as the output.

3.3.1 Optimal topology selection

Test experiments were conducted for different activation functions and the number of neurons in the hidden layer to obtain the optimal topology of the BP neural network. The test results are shown in Figure 7. The uncertainty of the test results was large because the initial weights and thresholds of the current BP neural network were randomly generated. Thus, the results of the test experiments were considered the average of 10 test trials. The learning rate of the training process of the BP neural network was 0.001, and the maximum number of iterations was 150.

Figure 7A shows the RMSE and R^2 of the three data sets with different numbers of neurons in the hidden layer under the condition that the activation function between the input layer and the hidden layer was Tansig. The RMSE of all three datasets roughly showed a tendency to decrease and then increase, and it reached the lowest point when the number of neurons in the hidden layer was 5. The R^2 of all three datasets showed a trend of increasing and then decreasing, reaching the highest point when

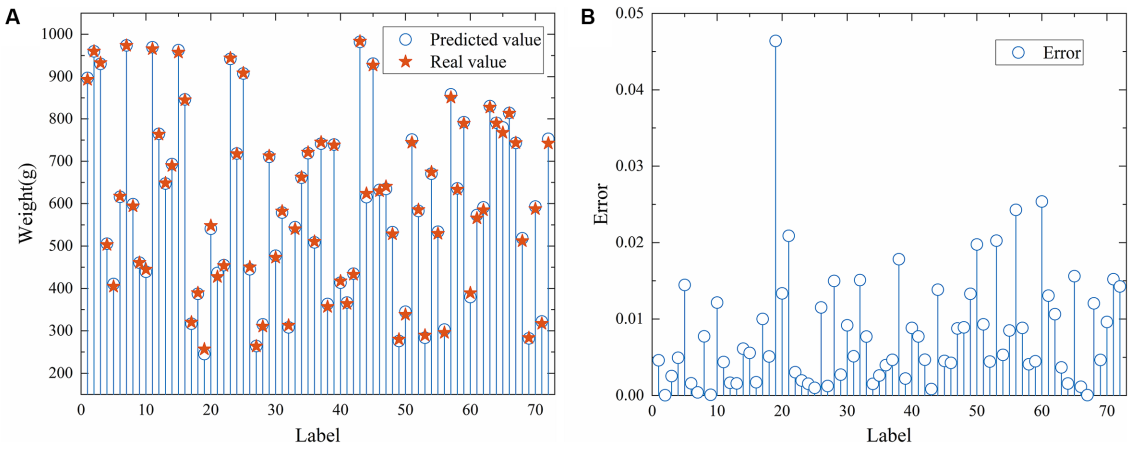

FIGURE 8
Test results for different topologies. The RMSE and R^2 of the three data sets with different numbers of neurons under the condition that the activation function were Tansig **(A)** and Logsig **(B)**.

the number of nodes in the hidden layer was 5. This finding was because of the inability of the topology of the neural network to satisfy the requirements of the training dataset when the number of neurons was extremely small, thereby reducing the model's accuracy. When the number of nodes was extremely high, the network was highly susceptible to overfitting, which decreased the model's accuracy (Yang et al., 2023). When the number of neurons in the hidden layer was 5, the RMSE and R^2 of the test set were 0.9844 and 25.3, respectively.

Figure 7B shows the RMSE and R^2 for three data sets with different numbers of neurons in the hidden layer. The activation function was Logsig between the input layer and the hidden layer. Figure 7B shows approximately the same pattern as Figure 7A, except that the optimal neuron number was 9, and the RMSE and R^2 of the test set were 0.9812 and 28.3, respectively. After comprehensively comparing the RMSE and R^2 for the two optimal neuron numbers, we obtained the optimal topology of the BP neural network with the activation function between the input layer and the hidden layer, which was Tansig. Moreover, the number of neurons in the hidden layer was 5.

3.3.2 Genetic algorithm optimization

After determining the optimal topology of the BP neural network, the model's training results were still unsatisfactory because the initial weights and thresholds of the neural network were randomly generated. This randomness results in local optimal training results, but not the global optimal. Figure 8 shows the training results after the genetic algorithm optimized the initial weights and thresholds of the BP neural network. The RMSE and R^2 of the test set were 4.9 and 0.9995, respectively, and the RMSE was reduced by 80.6% compared with the preoptimization. Figure 8A shows the fitting plot of the predicted and actual values of the test set, and the model had an excellent prediction effect. Figure 8B demonstrates the error plot of the test set's predicted and actual values. The maximum error within the permissible range of the online monitoring system for moisture content was 0.046.

3.4 Model performance comparison

Figures 9A–C show the scatter plots of the three datasets of PLSR, SVM, and BP neural networks, respectively. The scatter points of the BP neural network fusion model were more centrally distributed near the regression line than those of the two other models. The PLSR and SVM models showed satisfactory prediction results, but the RMSEs of the two prediction models were 41.4 and 39.2, which cannot satisfy the accuracy requirements of the online moisture content monitoring system. Although the structure and topology of the BP neural network are more complex compared to the other two models, collectively, the excellent prediction results of the BP neural network indicate that it can better capture the complex nonlinear relationship between the inputs and the outputs, and is also more suitable for online detection of moisture content. Each prediction model and structure has its applicable scenarios and limitations. Selecting a prediction model and structure depends on the problem and data characteristics in practical applications.

3.5 Validation experiment results and analysis

Table 2 shows the results of the validation experiments of the moisture content online monitoring system. The R^2 value of the nine sets of validation experiments was 0.9963, which was smaller than the R^2 of the weight fusion model (0.9995). A more significant uncertainty in the material moisture content detection than the material weight detection was observed. The moisture content detection based on the weighing method was based on the premise that the moisture content of the same batch of materials was the same. The samples used for the moisture content detection in the oven method always had error with the materials in the actual drying process, thereby increasing the material moisture content detection error. Nine groups of validation experiments had an RMSE of 0.78, and the maximum error was 6.27. The results of the validation experiments indicate that the online monitoring system of moisture content satisfies

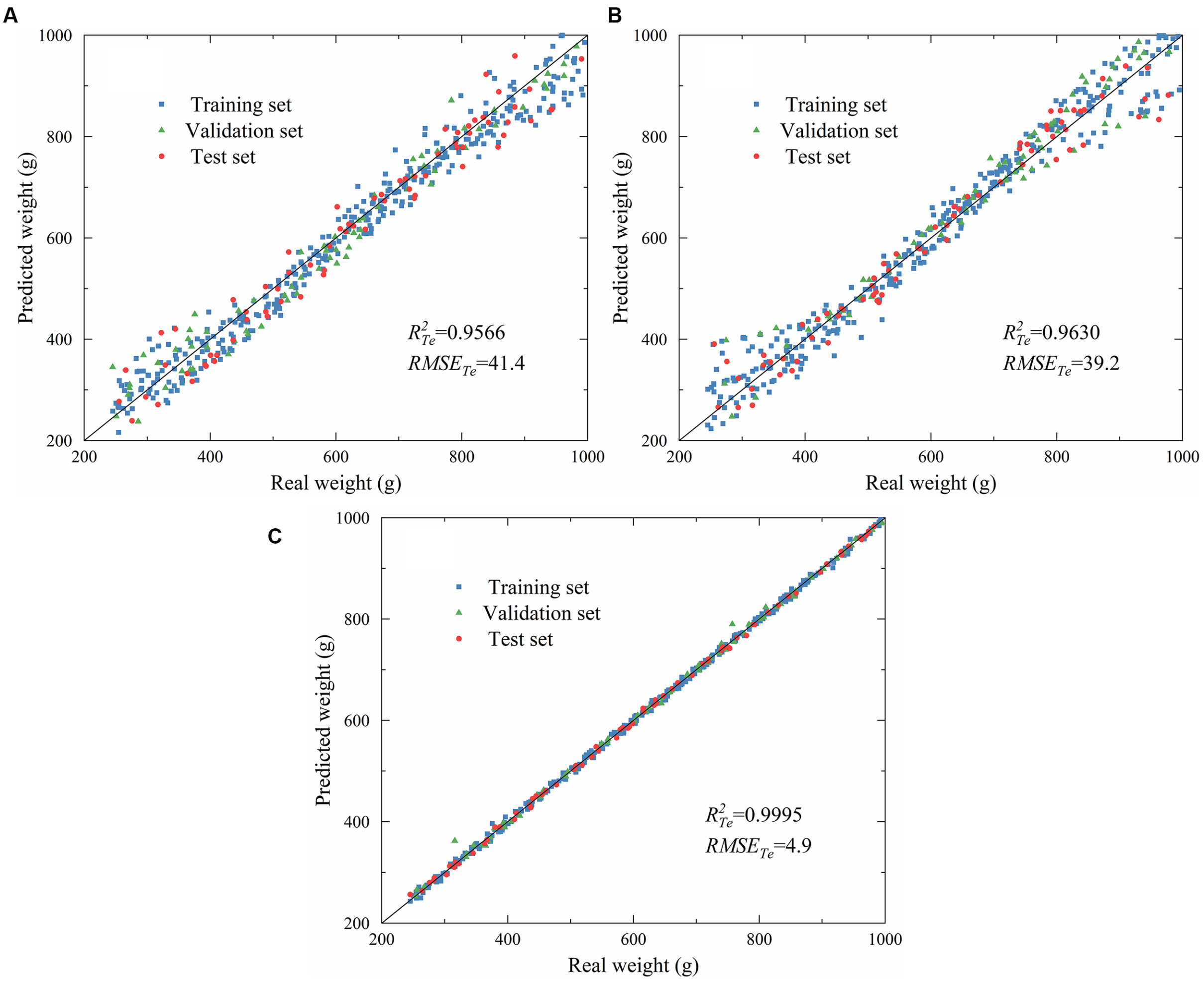

FIGURE 9
Comparison results of fusion model performance. The scatter plots of the three datasets of PLSR **(A)**, SVM **(B)**, and BP neural networks **(C)**.

TABLE 2 Results of validation experiments.

Groups	1			2			3			R^2	RMSE
Number	1	2	3	1	2	3	1	2	3		
True moisture content (%)	39.51	17.28	12.53	45.18	20.92	11.32	37.83	16.42	10.6	0.9963	0.78
Predicted moisture content (%)	38.48	17.89	13.21	44.42	20.09	11.99	38.62	17.45	10.98		
Error (%)	2.61	3.53	5.43	1.68	3.97	5.92	2.09	6.27	3.58		

the accuracy requirements of moisture content detection in the drying process.

4 Conclusion

An information acquisition system of material moisture content was developed, and the raw data used to establish the Kalman filter fusion algorithm were obtained. The Kalman filter processing was carried out on the raw detection values of the weight sensor and air velocity sensor, and the optimal state estimation of the weight detection value and air velocity was obtained. The information fusion processing of weight detection value, elastic substrate temperature, airflow velocity, and impingement distance was carried out using the estimated value, and the BP neural network fusion model with material weight as the output was established. The optimal topology of the BP neural network fusion model was selected; the activation function is Tansig, Pureline, and the number of neurons in the hidden layer is 5. The initial weights and thresholds of the BP neural network were optimized using genetic algorithms. The R^2 and RMSE of the test set of the optimized fusion model were 0.9995 and 4.9, respectively, and the RMSE was reduced compared with the preoptimization by 80.6%. Fusion models based on PLSR and SVM were also developed and compared with the BP neural network fusion model. The accuracy of the BP neural network model was significantly better than the two other models. An online moisture content monitoring system was constructed using the Kalman filter fusion algorithm, and validation experiments were carried out for the detection accuracy of the system. The R^2 and RMSE of the nine groups of validation experiments were 0.9963 and 0.78, respectively, indicating that the monitoring system satisfies the accuracy requirements of the material moisture content detection in the drying process. This study provides technical support for optimizing the drying process based on the change in moisture content, as well as a reference for online monitoring of moisture content in other drying equipment.

Data availability statement

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

Author contributions

TY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. XZ: Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Validation, Writing – review & editing. HX: Validation, Writing – review & editing. CS: Validation, Writing – review & editing. JZ: Validation, Writing – review & editing.

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Conflict of interest

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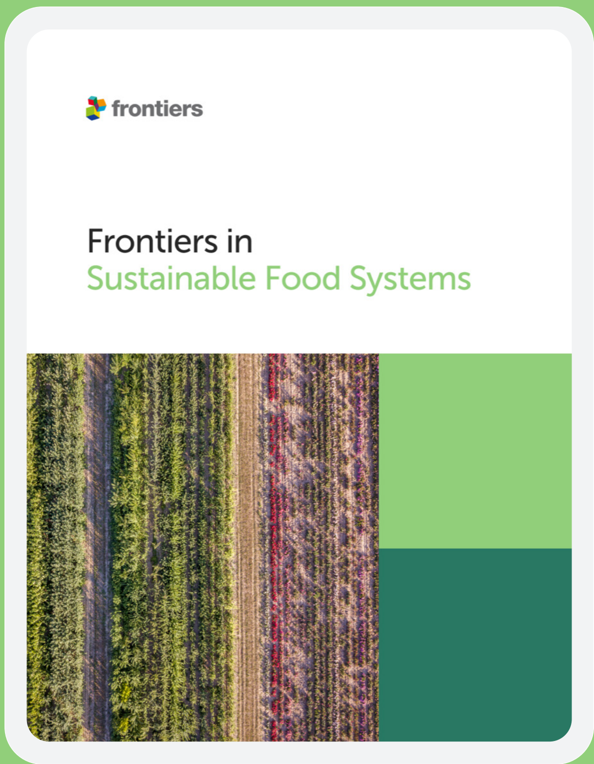

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