Journal of Food Quality

Biomaterials for Food Preservations

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Editorial

Biomaterials for Food Preservation

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Food, as such, is the most basic need of all life forms. However, its direct production is limited due to climatic conditions, limited resources, and skills. Thus, most postharvested produce are stored and transported from one region to another. Various agrochemical treatments were employed to maintain the quality characteristics and avoid damages to the postharvested food. These agrochemical preservative treatments provide safety, maintain quality, and long shelf life. However, due to their chemical nature, these have also exerted lethal health and environmental problems. Agrochemical residues in food have been recorded, and various health issues such as cardiac arrest, liver, kidney failure, and neurological disorders were also reported [1, 2]. Moreover, these residues are commonly escalated to the environment, mostly to water bodies, soil, and air, and raise various environmental and ecological issues [3]. This has resulted in a reduction of the acceptability of agrochemicals, which has led to the development of alternatives to agrochemicals. The use of biomaterials for postharvest quality preservation of foods has been reported as a significant means to provide an ecofriendly alternative to agrochemicals [4, 5]. These biomaterials commonly include chitosan, carboxyl methylcellulose, polysaccharides, alginate, and plant extracts, including essential oils, phytohormones, antioxidants, organic acids, and salts. The development and use of these biomaterials in the postharvest preservation of foods improve agricultural production sustainability, global food security, and environmental resilience.

The oils derived from plants may also have more useful properties beyond being just "edible." As such, essential oils extracted from the fruits and leaves of *Litsea cubeba* have been tested for antimicrobial properties. In an experimental and toxicity study, the inhibitory effects of *Litsea cubeba* oil, citral, citronellal, α-terpineol, and linalool were tested on *Aspergillus flavus* CGMCC 3.4408. Then, the effects of citral on the colony growth, the biomass, toxicity, and microstructure of *A. flavus* CGMCC 3.4408 were also investigated. The inhibitory effect of *Litsea cubeba* oil and its active components showed that citral had the best inhibitory effect on *A. flavus* 3.4408 growth. Furthermore, the inhibition of bacterial growth rate, biomass, and AFB1 synthesis showed the good bacteriostatic ability of citral to *A. flavus* strains isolated from moldy Chinese medicinal materials. Therefore, it should be encouraged to use citral as a mildew preventive agent for Chinese medicinal materials. Among fruits, pomegranate is rich in phenolic compounds and has the antioxidant capacity and the potential to inhibit free radicals. Besides its direct consumption as fruit and as juice, it has been tested to prepare cheese to provide a more nutritious and preservative effect. To produce Feta-type cheese with pomegranate juice, replacing part of the milk with pomegranate juice is necessary. The addition of pomegranate juice to the optimal whey-less Feta cheese changed its physicochemical and textural properties. The prepared cheese has better antioxidant properties and oxidative stability; however, its texture was weaker than the control and needed further study. Moreover, pomegranate and its peel waste exhibit excellent antimicrobial activity against several food-borne pathogens and improve the postharvest storability of food products. The high antioxidant activity, inhibition of lipid peroxidation, and

broad-spectrum antimicrobial efficiency of pomegranate peel play an intrinsic quality foundation for its development as a food preservative. Cinnamaldehyde (CA) is a major component of cinnamon essential oils extracted from the tree bark. Unlike many other natural compounds, cinnamaldehyde has been used as a potential antifungal agent, but its volatile nature and pungent smell limit its application. To mask this, multiple derivatives of cinnamaldehyde, namely, 4-nitro CA, 4-chloro CA, 4-bromo CA, 4-methyl CA, 4-methoxy CA, and 2,4-dimethoxy CA have been synthesized and tested for their antifungal potential against *Penicillium digitatum*, the major citrus fruit-rotting fungi. Among them, 4-methoxy CA showed the highest antifungal activity against citrus fruit postharvest-rotting fungi *Penicillium digitatum* (green mold). Moreover, 4-methoxy CA reduces the spore germination and growth by damaging the fungal cell membrane and declining the levels of reducing sugars. It appears that 4-methoxy CA is an excellent antifungal agent and can be used for postharvest storage of citrus fruits. Meat and meat products undergo quality loss due to oxymyoglobin and lipid oxidation and microbial growth. These phenomena cause adverse effects on the meat's nutritional, taste, color, and textural properties and thus need a natural preservative to meat. The effect of Shahpouri orange juice on lipid oxidation, color parameters, pH, and sensory properties of stacked and ground meat has been investigated. The incorporation of Shahpouri orange juice in stacked and ground meat decreased lipid oxidation due to antioxidant compounds and prevented decreasing redness. It was also revealed that the addition of Shahpouri orange juice at different levels could improve the sensory properties of meat. Considering these results, Shahpouri orange juice can be used as a natural additive in meat products to improve their quality during chilled storage. Organic acids are also used for the preservation of meat products. They inhibit or delay a wide range of spoilage and pathogenic microorganisms, improve the physicochemical properties of meats, and improve their organoleptic attributes (stability of red color, enhancement of flavour, suppression of rancidity and undesirable off-odor, inhibition of slime formation and gas production, and improvement of tenderness and juiciness). Besides these excellent properties, the use of organic acid for meat preservation is limited due to several factors implicating organic acid/salt characteristics (form, concentration, and antimicrobial activity), meat (type, composition, microbial loads, pH, and A_w), and environmental conditions (temperature, humidity, storage, and packaging). In addition, extensive use of organic acids could result in bacterial adaptation, making some microorganisms acid-tolerant and resistant in other ways, which represent challenges for their use as food preservatives, particularly in meats. Combining organic acids/salts with other control or preservative techniques (e.g., chemical, physical, and biological) needs to be tested based on potent synergistic activities or additional effects that could effectively reduce the challenges and improve the preservation properties and quality of the stored food. The food itself contains many preservative chemicals and identifying such novel natural chemicals is in demand. As such, the bioactive antioxidant peptides from potato protein hydrolysate have been identified. Three prominent peptides, namely, Phe-Tyr, Tyr-Phe-Glu, and Pro-Pro-His-Tyr-Phe, which matched the sequence of patatin and were made up of Phe and Tyr, were identified. Furthermore, the peptide Tyr-Phe-Glu demonstrated higher antioxidant activity against Caco-2 cell oxidation induced by H_2O_2. These identified bioactive peptides could be further used as a biomaterial agent for food preservation. Nonbiodegradable and synthetic polymeric substances are vastly used as food packaging materials that have caused significant environmental concerns. In contrast to synthetic and semisynthetic polymers, fruit polysaccharides are for sure vastly available, cheap in processing costs, nontoxic, biodegradable, and not dangerous to the environment, and these features make them be applied more rather than synthetic agrochemicals. Lastly, *Cordia* species has been investigated for its effects on apple jelly's physicochemical, color, textural, rheological, microstructural, and sensory properties. The results showed that gum cordia had a significant effect on apple jelly's physicochemical properties (ash, protein, phenolic content, degree of esterification, and color). The total phenol content of the sample significantly increased with the addition of gums. The rheological properties showed that a sample containing 75% gum cordia was similar to control and had the highest apparent viscosity, loss moduli (G''), storage moduli (G'), and complex viscosity. Also, the sensory properties showed that a sample containing 75% gum cordia had a high score in texture, taste, appearance, and overall acceptability. These results suggested that gum cordia as a polymer can be successfully employed to formulate jelly and further improve the technofunctional properties of jelly, thus improving the storage properties.

In conclusion, this special issue provides advances in biomaterials research for food preservation, specifically on the identification, synthesis, and applications of biomaterials. Their prospective use in food preservation has the potential to lead towards the production of safer and healthier foods, improve the postharvest quality attributes, and provide a long shelf life. Besides these, the use of biomaterials for food preservation would also limit environmental toxicity by replacing toxic synthetic chemicals.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this special issue.

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Chunpeng (Craig) Wan
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Chuying Chen

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Review Article

Chemistry, Safety, and Challenges of the Use of Organic Acids and Their Derivative Salts in Meat Preservation

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Meat industries are constantly facing new waves of changes in the consumer's nutritional trends, food safety, and quality requirements and legislations leading to an increase in interest for meat biopreservation to respond to all of these modern socioeconomic demands. Hence, to replace synthetic and/or expensive additives, new technologies in preserving meat products from microbial contamination have been established. In this context, organic acids and their salts have been considered as the most popular examples of preservatives that offer several advantages to be applied in meat industry. Here, characteristics of organic acids/salts commonly used in meat preservation were described based on the published literature. Moreover, after outlining the challenges and advantages of their use in meat industry, their current applications as meat preservatives on various meat type matrices such as beef, pork, sheep, and poultry were quite exposed based on previous and recent research works. Then, different application types were highlighted. Besides, some potent synergistic approaches based on several combinations of organic acids/salts with different existing preservative techniques are reported with an emphasised discussion of their application as possible solution tools to mainly overcome some problems linked to organic acids/salts when used solely, thus contributing to ensure the overall safety and improve the quality of meats. Finally, despite their usefulness in meat preservation, organic acids/salts may possess detrimental traits. In this context, a detailed discussion on their limits of use in meat products was provided in the last section of this paper.

1. Introduction

Food preservation represents an important industrial challenge in terms of preserving nutritional and organoleptic qualities and food safety. In addition, consumer desires ranging from freshness, long-term storage, absence of chemical preservatives, and reasonable price of foods. To respond to all of these requirements, several preservation methods including drying, freezing, refrigeration, thermal processing, irradiation, modified atmosphere packaging (MAP), and addition of antimicrobials have been employed [1, 2]. However, despite the use of these various techniques, many issues are still encountered in terms of food spoilage, thus resulting in economic loss at industry level scale, and consumer health and, consequently, leading to diseases ranging from short-lived to severe complications and even death.

On the other hand, there is an increasing demand from consumers for more natural foods over the last decade that raises a great interest towards food biopreservation. This modern movement leaded to the search and development of new antimicrobials from various natural (animal, plant, and microbial) origins [3, 4]. Among the more widely researched antimicrobials as biopreservatives in food systems are organic acids and their derivative salts. Lactic, acetic, and citric acids and their corresponding salts have been of considerable interest since they occur naturally in foods. In addition, these preservatives exhibit antimicrobial activity by

inhibiting the growth of several spoilage and pathogenic microorganisms, improve the sensory properties, and possess technological functions such as the stabilisation of colour, regulation of acidity, and development of characteristic flavour of several foods [5, 6]. Indeed, many organic acids are generally recognised as safe (GRAS) antimicrobials that could be used in food industry [6–8]. In this paper, we will focus on the most known ones commonly applied in meat biopreservation since these foods are assigned as very perishable due to their very short shelf-life because of microbial spoilage and lipid oxidation [5, 9]. In this line, we could notice that, in meat and meat products, the major challenges are the prevention of harmful microorganisms' growth and preservation of sensory properties (colour, flavour, odour, texture, etc.). Therefore, to successfully attain these objectives as well as responding to modern consumer trends and food legislation related to meat biopreservation, organic acids and their derivative salts seem to be very good examples of natural preservatives.

In this context, the present paper presents a general overview on organic acids and their salts in terms of their usefulness and practical importance as biopreservatives in meat. In fact, this review highlights their structures and aspects, antimicrobial action, roles in safety assurance, shelf-life prolongation, and organoleptic quality enhancement basing on the previous and recently published research and studies. Finally, some powerful approaches and promising strategies are exposed to potentially enhance the preservative effect of organic acids/salts in meat preservation and satisfy both meat industry's demand and consumer's desire for natural, safe, healthy, and low-cost meats (at production as well as at market purchase). Hence, the pros and cons of these natural tools are deeply discussed, and by this review, we hope to provide a valuable addition to mainly contribute to the enrichment of the scientific library and help any researcher working in this field.

2. General Characteristics of Organic Acids

2.1. Nature, Composition, and Production. Organic acids are chemical compounds widely present in nature as normal constituents of plants or animal tissues [10]. For instance, citric acid can be extracted from the juice of citrus and other acidic fruits such as limes, lemons, oranges, pineapples, and gooseberries [11–13], while benzoic acid exists naturally in cinnamon, cloudberries, cranberries, and lingonberries [3]. Ubiquitous microorganisms such as bacteria, fungi, and yeasts can also produce organic acids [10]. Nevertheless, lactic acid bacteria (LAB) are the most popular bacteria regarding the production of organic acids as their metabolic end-products of carbohydrate fermentation [3, 14]. Hence, these acids could occur naturally as ingredients of several food products or could later be added to them. Otherwise, organic acids possess the common characteristic of having carbon in their structure. Those having 10 carbons or less could thus be distinguished from fatty acids which have in their structure straight and even-number carbon chains of 4 to 24 [3]. In general, organic acids are weak acids and do not dissociate completely in water. Organic acids with low molecular mass such as lactic and formic acids are miscible in water; however, those with high molecular mass, such as benzoic acid, are insoluble in molecular form [6]. Furthermore, organic acids are mostly known to be very soluble in organic solvents [5]. On the other hand, it is important to note that organic acids exist in two basic forms: (i) pure organic acids (e.g., lactic, acetic, citric, and benzoic acids) and (ii) buffered organic acids such as the calcium and sodium salts of the already mentioned acids [3]. Organic acids differ in structure basing on the involvement of their constituent elements of carbon, hydrogen, and oxygen [10]. Chemical properties and physical characteristics of the most used organic acids and their salts that are commonly used in food industry are presented in Table 1.

2.2. Antimicrobial Activity and Mode of Action of Organic Acids and Their Salts

2.2.1. Antimicrobial Activity. Thanks to their potential antimicrobial activity widely reported in several studies, organic acids and their salts have gained a renewed interest to be applied as bioagents in meat industry. In fact, these acids and their salts have been demonstrated to exhibit a large spectrum of action against Gram-positive bacteria [5, 15–17], Gram-negative bacteria [15–19], and fungi and yeasts [20–22]. Some of the antimicrobial effects of common and most studied organic acids and their salts are summarised in Table 2.

2.2.2. Mode of Action. In general, the bioactive agents act as bacteriostatic or bactericidal molecules towards microorganisms suggesting several antimicrobial mechanisms of action [35–37]. The antimicrobial activity of organic acids such as lactic, acetic, and citric acids and/or their salts is based on lowering the pH level of a medium or foodstuff as a mechanism of action to inhibit the microbial growth [6, 38–40]. This pH decrease ability is dependent upon the chemical properties of organic acid compounds such as the acid constant (pK_a) and the dissociation constant (K_a) numbers (Table 1), the concentration of the undissociated forms as well as the organic acid concentration [6, 41]. In fact, the organic acids exist in a pH-dependent equilibrium between the dissociated and undissociated states [3]. The undissociated and uncharged organic acid molecules are first responsible for the antimicrobial activity since their concentration increases at low pH [3]. For this reason, it was mainly noticed that organic acids have optimal antimicrobial effects at low pH [3]. Here, it was believed that these undissociated molecules are lipophilic and therefore are able to easily cross the lipidic membrane of target microorganisms (bacteria, yeasts, and fungi) to enter into their cytoplasm, thus resulting in cell death [6, 42]. Indeed, once inside the cytoplasm of the bacterial or fungal cell, the organic acid molecules encounter a higher pH, provoking then their dissociation into charged anions and protons that could not recross the plasma membrane [3, 6, 42]. The accumulating H^+ ions will then decrease intracellular pH toward harsh physiological conditions inducing cellular damage and

Table 1: Characteristic of the most known organic acids and their salts.

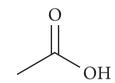
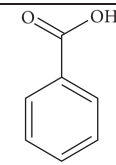
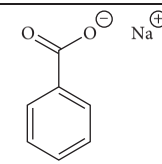
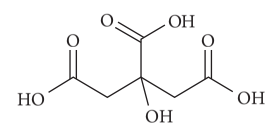
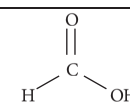
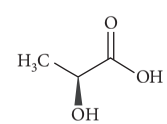
Organic acid/salt	Origin			Molecular formula	Molecular weight (g/mol)	Structural formula	pK_a	K_a	Physical appearance	Solubility in water (g/L)
	Microbial producer	Plant	Animal							
Acetic acid	*Acetobacter* spp., *Komagataeibacter europaeus*, *Dekkera bruxellensis*, *Brettanomyces* spp., *Saccharomyces cerevisiae*, *Fusarium oxysporum*, *Polyporus anceps*	—	—	$C_2H_4O_2$	60.05		4.76	1.73×10^{-5}	Liquid	Miscible
Benzoic acid	—	—	—	$C_7H_6O_2$	122.12		4.2	6.3×10^{-5}	Solid	2.9
Sodium benzoate	—	Cranberries, bilberries	—	$C_7H_5NaO_2$	144.10		4.2	6.3×10^{-5}	Solid	660
Citric acid	*Aspergillus*, *Acremonium*, *Ascochyta*, *Bacillus*, *Botrytis*, *Candida*, *Debaryomyces*, *Eupenicillium*, *Hansenula*, *Mucor*, *Penicillium*, *Pichia*, *Saccharomyces*, *Talaromyces*, *Torulopsis*, *Trichoderma*, *Yarrowia*, *Zygosaccharomyces*	All citrus fruits (lemons, limes, oranges), pineapples, gooseberries	—	$C_6H_8O_7$	192.12		3.13; 4.76; 6.4	7.4×10^{-4} 1.7×10^{-5} 3.9×10^{-7}	Solid	750
Formic acid	—	*Urtica dioica*	Ants, bees	CH_2O_2	46.025		3.745	1.8×10^{-4}	Liquid	Miscible
Lactic acid	*Aspergillus*, *Bacillus*, *Carnobacterium*, *Enterococcus* sp., *Escherichia*, *Lactobacillus plantarum*, *Lactococcus* sp., *Leuconostoc mesenteroides*, *Rhizopus*, *Saccharomyces*, *Candida*, *Pichia stipites*, *Torulaspora delbrueckii*	—	—	$C_3H_6O_3$	90.07		3.9	1.3×10^{-4}	Liquid	Miscible

Table 1: Continued.

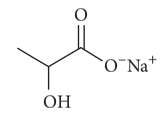
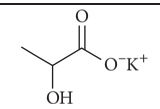
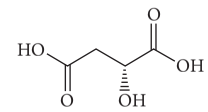
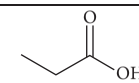
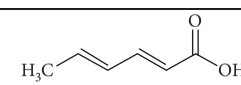
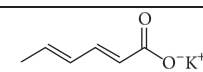
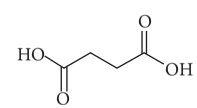
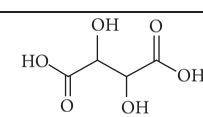
Organic acid/salt	Origin			Molecular formula	Molecular weight (g/mol)	Structural formula	pK_a	K_a	Physical appearance	Solubility in water (g/L)
	Microbial producer	Plant	Animal							
Sodium lactate	—	—	—	$C_3H_5NaO_3$	112.06		3.9	1.3×10^{-4}	Liquid (colourless or slightly yellowish)	Miscible
Potassium lactate	—	—	—		128.17		3.9	1.3×10^{-4}	Liquid (colourless, clear)	Viscous
Malic acid	*Aspergillus* spp., *Escherichia*, *Saccharomyces*, *Zygosaccharomyces*	Blackberries, blueberries cherries, apricots, peaches, mango, plums, apples, pears, quinces, mirabelles	—	$C_4H_6O_5$	134.08		3.46; 5.10	—	Solid	558
Propionic acid	*Lactobacillus* spp., *Clostridium propionicum*, *Propionibacterium* spp.	Apples, grains, strawberries	—	$C_3H_6O_2$	74		4.87	1.3×10^{-5}	Liquid	370
Sorbic acid	—	Berries of rowan tree (*Sorbus aucuparia*)	—	$C_6H_8O_2$	112.13		4.76	1.73×10^{-5}	Solid	1.6
Potassium sorbate	—	—	—	$C_6H_7KO_2$	150.21		4.76	1.73×10^{-5}	Solid	1400
Succinic acid	*Anaerobiospirillum succiniciproducens*, *Mannheimia succiniciproducens*, *Actinobacillus succinogenes*, *Bacillus fragilis*, *Fusarium* spp., *Aspergillus* spp., *Candida* spp., *Yarrowia lipolytica*, *Saccharomyces cerevisiae*	—	—	$C_4H_6O_4$	118.088		4.2 5.6	6.21×10^{-5} 2.31×10^{-6}	Solid	58
Tartaric acid	*Gluconobacter* spp.	Grapes, bananas, tamarinds, citrus	-	$C_4H_6O_6$	150.08		2.89 4.40	6.8×10^{-4} 1.2×10^{-5}	Solid	1330

Table 2: Spectrum of action of frequently encountered organic acids and their salts.

Organic acid/salt	Activity spectrum	Reference
Lactic acid	*L. monocytogenes*, *S. aureus*, *E. faecalis*, *B. cereus*, *Salmonella* sp., *E. coli*, *P. aeruginosa*, *Proteus* sp., *C. albicans*, *S. cerevisiae*, *Penicillium nordicum*, *Penicillium purpurogenum, Aspergillus flavus*, *Rhizopus nigricans, Rhodotorula* sp.	[5, 17–21, 23–25]
Sodium lactate	Psychrotrophic bacteria, faecal streptococci, *L. monocytogenes*, *Enterobacteriaceae*, *E. coli, Salmonella* sp.	[26]
Potassium lactate	*L. monocytogenes*, *E. coli, Salmonella* sp.	[26]
Citric acid	*L. monocytogenes*, *Salmonella typhimurium*, *E. coli* O157:H7, *Aspergillus flavus*, *Penicillium purpurogenum, Rhizopus nigricans, Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*	[5, 18, 19, 21, 24, 27, 28]
Sodium citrate	*Fusarium* sp.	[21]
Acetic acid	*L. monocytogenes*, *E. coli* O157:H7, *Salmonella typhimurium*, *Enterobacteriaceae, Penicillium nordicum*, *Penicillium purpurogenum, Aspergillus flavus, Rhizopus nigricans, Fusarium* sp.	[5, 18–21, 23–25]
Propionic acid	*L. monocytogenes, E. coli, Salmonella* spp., *Clostridium perfringens, Aspergillus flavus, Fusarium* sp., *Penicillium* sp., *Rhizopus nigricans,*	[16, 19, 21, 23, 24]
Tartaric acid	*Salmonella typhimurium, Aspergillus flavus, Fusarium* sp., *Penicillium* sp., *Rhizopus nigricans*	[16, 18, 21]
Formic acid	*E. coli, Salmonella* spp., *Clostridium perfringens, Aspergillus flavus, Fusarium* sp., *Penicillium* sp., *Rhizopus nigricans*	[16, 21]
Sodium formate	*Streptococcus* sp., *Clostridium perfringens, E. coli, Salmonella enterica* typhimurium, *Campylobacter jejuni*	[15]
Benzoic acid	*E. coli*, *L. monocytogenes*	[29]
Sodium benzoate	*Fusarium sambucinum, L. monocytogenes*	[21, 30–32]
Succinic acid	*Salmonella typhimurium*, *E. coli, B. subtilis, S. suis*	[19]
Sorbic acid	*Fusarium* sp., *L. monocytogenes*, *E. coli*	[21, 29, 33]
Potassium sorbate	*Fusarium* sp., *L. monocytogenes*, *Salmonella* spp.	[21, 30, 31, 34]

modification of the functionality of enzymes, structural proteins, and DNA [43, 44]. Furthermore, the generated anions were demonstrated to be toxic leading to inhibit metabolic reaction and cause cell death [3, 44]. On the other hand, the release of these anions increases the osmotic pressure in the cytoplasm that is deleterious to cytosolic enzymes [44]. This mechanism of action is generally attributed as "weak organic acid theory" [43]. In this line, it was previously reported that weak organic acids such as sorbic and benzoic acids could act in their undissociated forms with penetrating microbial cells and inactivating them by lowering their internal pH level or by interfering with metabolic reactions [7]. Therefore, the organic acids were assigned to exhibit stronger antimicrobial effects than the highly dissociating inorganic acids at the same pH level due to their amount of undissociated molecules and their cell penetration capacity, suggesting their application in food biopreservation.

Other modes of action were suggested for the microbial growth inhibition by weak organic acids including induced stress response on intracellular pH homeostasis and cell membrane disruptions related to proton motive force and membrane transport resulting in energy depletion and inhibition of essential metabolic reactions [3, 42, 45]. Effectively, target affected by organic acids attempts to counter the unsuitable cellular acidification by restoring homeostasis using an integral membrane protein known as Hsp30 to actively export the excess proton out of the cytoplasm. This stress response leads to a rapid starvation, thus reducing the cellular energy required for growth and several metabolic functions that were already consumed for protein activity in maintaining homeostasis [6, 46]. Moreover, the cell electrochemical potential dropped, and nutritional substance uptake was inhibited [6]. These mechanisms were observed for lactic, malic, citric, acetic, and propionic acids although the classical "weak organic acid theory" as mode of action has previously been attributed to them [6, 42, 47, 48]. For instance, lactic acid was shown to destabilise the membrane of Gram-negative bacteria by the reduction of membrane-associated molecular interactions resulting in the formation of pores which causes rapid cell death [49]. Furthermore, lactic and acetic acids were found to disturb the transmembrane proton motive force, denature acid-sensitive proteins and DNA, and overall interfere with both metabolic and anabolic processes [42]. Regarding citric and malic acids, they were able to destabilise the cellular outer membrane by intercalation or chelation of essential ions, thus inhibiting their access for the cell, and then, metabolic pathways that depend upon certain ion conditions will be arrested [6, 28, 44]. Concerning sorbic acid, it may not only act as a classic weak organic acid but also could inhibit the membrane-associated protein H^+-ATPase that is implicated in the microbial respiration chain crucial for cellular energy generation [43]. On the other hand, according to previous results of York and Vaughn [50], another mode of action of sorbic acid, involving the inhibition of intracellular enzymes (e.g., cysteine) by interactions with their thiol groups, was proposed.

Therefore, it is worth mentioning that weak organic acids do not display one basic mechanism of action as already mentioned, and deep investigations of these modes of action remain to be continued to clearly explain the outcomes in this regard in terms of target sites, action on bacteria, moulds and/or yeasts, and interactions with the surrounding environment.

2.3. Other Technological Functions of Organic Acids. Many organic acids and their salts are GRAS and approved to be applied in meat industry for different matrices thanks to their various and useful effects [6]. In addition to their potential antimicrobial activity towards several spoilage and pathogenic microorganisms, organic acids and their salts display further functionalities helpful for their application as biotechnological agents such as antioxidant activity [51–53], enhancement of physicochemical properties, and improvement of sensory attributes of meat and meat products [54–57]. In this context, these acids and their salts were accordingly listed in the European legislation as preservatives (P), acidifiers (A), acidity regulators (AR), antioxidants (AO), stabilisers (S), colour stabilisers (C), flavour enhancers (FE), and flavour treatment agents (F) [8]. Table 3 highlights approved technological functions of some organic acids and their salts commonly used in food industry with a close link to meat biopreservation purpose.

2.4. Regulatory Use of Organic Acids and Their Salts as Food Preservatives. Regarding the regulation for use of organic acids and their salts as food preservatives in meat, it is evident that each compound must technologically be applied in different meat matrices at specified concentrations. This latter must be respected to a legislative values determined from the Acceptable Daily Intake (ADI), in which the maximum, corresponding to a hundredth of the No Observable Adverse Effect Level (NOAEL), must not be exceeded [6]. The ADI values for the most common organic acids and their salts used as preservatives in meat are shown in Table 4. Generally, the maximum applicable amounts of the majority of organic acids and their salts in foodstuffs range between 0.1 and 0.4% [6]. Specifically, in accordance with European food legislation, lactic, citric, and acetic acids should be applied with *quantum satis* principle (no maximum level is specified, but the applied amount shall be used at a level not higher that is necessary to achieve the intended technological purpose) [8]. Regarding sorbic and benzoic acids, the allowed amounts normally range between 1000 and 2000 mg/kg of food [8]. Details related to toxicological data, ADI values, and application limits of some organic acids and their salts are given in Table 4.

3. Application of Organic Acids and Their Salts in Meat Preservation

The use of synthetic preservatives in meats such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) remains a major issue since these chemical additives have been involved in several health problems [58]. Therefore, as a challenge, meat industries and scientific researchers have increased their attention on investigating natural antimicrobials efficient in preserving meats in terms of preventing their microbial, physicochemical, and sensory deteriorations during processing, storage, and transport and extending their shelf-life in accordance with food legislation. In this line, the application of organic acids and their salts gained a great attention as food preservatives in meat and meat products since they are food ingredients and often naturally produced by several microorganisms. But, despite the wide numbers of useful and well characterised organic acids and their salts for their efficiency as preservatives, until now there are likely still relatively few ones coming onto natural agents approved for such application in meat industries. Effectively, strict *in vitro* and *in vivo* assessments must be performed before concluding about their effectiveness in preserving different meat systems to finally obtain approval from the international regulation agencies. These evaluations could thus take many years because of their complexity in terms of ADI and NOAEL determination to avoid allergenic and toxicological problems when consumed, high antimicrobial and preservative activities when incorporated into diverse meat matrices, absence of effects on the meat sensory properties, and absence of interactions with meat components.

The worldwide policy in the agrofood field is the implementation of improved meat quality and safety with an emphasised focus on the most perishable foodstuffs such as meats and derivative products. Although organic acids have been previously used for such purposes, there is a glaring need to evaluate and improve their continued efficiency and sustainability.

3.1. Advantages of Organic Acids and Their Derivative Salts Application in Meat and Meat Products. Recently, it was demonstrated that organic acids and their salts have been of considerable value as natural preservatives of meat and meat products and their application represent a promising biotechnological approach. In fact, their use is quite advantageous as they have GRAS status since they are natural food ingredients and several microorganisms (e.g., LAB, yeasts, and fungi) naturally produce most of them [6]. Hence, due to this great and natural distribution, their extraction seems to be much easier and simple comparing to other rare antimicrobials. Another advantage of the use of organic acids and their salts in meat and meat products is the fact that they did not affect the organoleptic quality when applied in respect to amounts allowed by the food legislation. Moreover, most of them are not limited in the ADI for humans such as lactic, citric, and acetic acids [8, 19].

Furthermore, according to the published literature, many of the characterised organic acids and their salts have shown potent antimicrobial activity with large spectrum of action against spoilage and pathogenic microorganisms (Table 2) with significant improvement of the physicochemical (delay of lipid and protein oxidation) and sensory properties (stabilisation of red colour, inhibition of off-odour, and enhancement of characteristic flavour or taste or tenderness or juiciness) of treated meats, suggesting that

Table 3: Technological functionality of commonly used organic acids and their salts in food industry.

E. no	Organic acid/salt	Functionality						
		Preservative	Acidifier	Acidifier regulator	Antioxidant	Stabiliser	Colour stabiliser	Flavour enhancer
E200	Sorbic acid	×						
E202	Potassium sorbate	×						
E210	Benzoic acid	×						
E211	Sodium benzoate	×						
E212	Potassium benzoate	×						
E260	Acetic acid	×	×	×				
E261	Potassium acetate	×		×				
E262	Sodium acetate	×		×				
E270	Lactic acid	×	×				×	
E280	Propionic acid	×						
E296	Malic acid		×				×	
E300	Ascorbic acid		×		×		×	
E301	Sodium ascorbate		×		×		×	
E325	Sodium lactate			×		×		
E326	Potassium lactate			×		×		
E330	Citric acid		×				×	
E334	Tartaric acid		×				×	
E355	Adipic acid		×					×
E363	Succinic acid		×	×				

Table 4: Lethal dose by rat oral ingestion (LD_{50} rat), Acceptable Daily Intake (ADI), and application limits in meat of commonly used organic acids/salts.

Organic acid/salt	LD_{50} rat, oral (mg/kg_{BW})	ADI (mg/kg_{BW})	Application limits in meat
Acetic acid	3310	Undefined	*Quantum satis*
Sodium acetate	3530	Undefined	Good manufacturing practice
Benzoic acid	1700	5	1000 ppm
Sodium benzoate	4070	5	1000 ppm
Citric acid	3000	Undefined	*Quantum satis*
Lactic acid	3540	Undefined	*Quantum satis*
Potassium lactate	>2000	Undefined	Good manufacturing practice
Propionic acid	370	Undefined	2500 ppm
Tartaric acid	7500	30	Good manufacturing practice
Sorbic acid	7360	25	1000 ppm
Potassium sorbate	2600	25	1000 ppm

LD_{50} rat: lethal dose 50% for rat, dose required to kill 50% of tested rats; oral: oral ingestion; BW : body weight.

these bioagents could have important functions as natural meat preservatives.

Finally, it is important to denote that the application of organic acids and their salts in meat preservation become nowadays common because acid treatment procedures are cheap, simple, and fast to apply [19].

Therefore, taken into account all of these potential aspects and merits played by organic acids and their salts, it could be admitted that they are attracting considerable interest which favour their use as innovative natural food preservatives to ensure safety, improve quality, and prolong the shelf-life of meats.

3.2. Current Applications of Organic Acids and Their Salts in Meat Preservation

3.2.1. Beef Meat. Added to vacuum-packaged raw ground beef at 30 g/kg, sodium lactate showed an interesting result on the microbiological and chemical qualities [59]. In fact, its addition significantly delayed the proliferation of aerobic plate counts, psychrotrophic counts, LAB, and Enterobacteriaceae and clearly extended the shelf-life of the treated beef up to 15 days *versus* 8 days only for control. In addition, over the storage experiment (21 days at 2°C), this organic salt (sodium lactate) maintained the ground beef at almost constant pH and lipid oxidation was not affected.

Furthermore, it has been reported by Quilo et al. [60] that the use of potassium lactate at 3% on beef trimmings before grinding could improve the sensory (odour and taste) attributes and technological (cooking yield) characteristics and maintain the physicochemical (pH and TBARS) parameters.

Another study of the effect of sodium and potassium lactates at 2% on the cook yield and tenderness of bovine chuck muscles highlighted a significant ($P < 0.05$) decrease of the WBSF (Warner–Bratzler shear force) and a remarkable improvement of sensory attributes (tenderness

and cook yield rating) compared to noninjected controls [61].

On the other hand, lactic and acetic acids at 2% displayed strong ($P < 0.05$) antimicrobial activity on the decontamination of *E. coli* O157:H7 onto beef meat surfaces according to Carpenter et al. [62]. In fact, these organic acid washes decreased the pathogen by 0.6 to 1 log/cm^2 compared to control samples (no-wash meats) or water-wash meat samples and prevented its residual growth.

These same organic acids (lactic and acetic acids at 1 and 2%) and their salts (sodium lactate and sodium acetate at 2.5%) were assessed for their effects on the chemical, microbiological, and sensory qualities of raw beef meat stored at 4°C [63]. Results revealed the efficient antimicrobial activity of these additives against the proliferation of aerobic and psychrotrophic bacteria as well as Enterobacteriaceae. The chemical analysis showed a significant ($P < 0.05$) reduction in the pH of treated beef samples. Regarding the sensory quality of treated beef meats, the organic acids/salts addition improved the colour, flavour, and texture properties with highest scores shown for 1% acetic acid and 1% lactic acid.

In addition, it was reported that 2% potassium lactate could ensure microbial quality of roast beef towards the proliferation of *L. monocytogenes* and inoculated *Salmonella* cocktail composed by *Salmonella typhimurium*, *Salmonella* Heidelberg, and *Salmonella* Enteritidis [64].

A more recent research [65] indicated that lactic, acetic, and citric acids at 1, 2, and 3% reduced *Salmonella* Enteritidis, *E. coli*, and *L. monocytogenes* counts in beef meat. Significant ($P < 0.05$) reductions were observed with 3% concentration, and the most efficient organic acid was lactic acid, followed by acetic acid and citric acid.

Moreover, regarding lactic and acetic acids at 3%, it has been found that their spraying on bovine carcasses significantly ($P < 0.05$) decreased the counts of spoilage psychrotrophic germs and pathogenic bacteria (*Pseudomonas* spp., *Aeromonas* spp., *Yersinia* spp., and Enterobacteriaceae) during 14 days [66]. The most sensitive bacteria towards the lactic and acetic acids were *Aeromonas* spp.

3.2.2. Pork Meat. The addition of sodium lactate at 0, 1, 2, and 3% was found to depress aerobic total plate counts, anaerobic total plate counts, and anaerobic lactic acid producers on vacuum-packaged pork sausages stored at 4°C for 31 days [67]. In addition, this organic salt at 1% extended the shelf-life of pork sausages by 1 week compared to controls, and samples containing 2 and 3% of sodium lactate had not reached spoilage level ($\log_{10}$ 7.0 CFU/g). Regarding its effects on physicochemical characteristics, sodium lactate reduced the pH of pork samples. On the other hand, it was found that the addition of sodium lactate generally increased salty taste and prevented the loss of pork flavour over time but had no effect ($P > 0.05$) on sour or bitter flavours.

Further study was performed with sodium lactate at 1.5 and 3% to evaluate its effects on the sensory characteristics and shelf-life of fresh ground pork [68]. As a result, the sodium lactate increased the salty flavour intensity but remained less perceptible as "salty" than the other salt additive of NaCl. It also increased juiciness and enhanced ground pork flavour. Additionally, sodium lactate significantly ($P < 0.05$) reduced aerobic plate counts in fresh ground pork over the retail storage of 25 days, and meat samples formulated with 3% sodium lactate exhibited a great extending of shelf-life by about 12 days compared to controls.

Similarly, it has been found that packaged ground pork meat, aerobically stored at 4°C for 21 days and formulated to contain 3% of sodium lactate, had the lowest aerobic plate counts and its initial pH was best maintained [69]. Moreover, meat red colour was best preserved by 2 and 3% of sodium lactate concentrations.

On the other hand, acetic or lactic acids at 2.5 or 5% and some organic salts (sodium acetate, sodium diacetate, sodium lactate, potassium sorbate, and potassium benzoate) at different concentrations (5 or 10%) were used as aqueous dipping to control *L. monocytogenes* on sliced and vacuum-packaged pork Bologna stored at 4°C for up to 120 days [31]. Results showed no significant ($P > 0.05$) growth of *L. monocytogenes* on pork Bologna slices treated with 2.5 or 5% acetic acid, 5% sodium diacetate, and 5% potassium benzoate from days 0 to 120. Meat products treated with 5% potassium sorbate and 5% lactic acid were stored for 50 and 90 days, respectively.

In addition, sodium lactate, sodium acetate, and sodium diacetate were previously assessed for their antimicrobial activity against *L. monocytogenes* growth on frankfurters stored at 4°C in vacuum packages [70]. The results revealed that 6% sodium lactate and 0.5% sodium diacetate had bactericidal effects for 120 days. Then, sodium lactate at 3% prevented listerial growth for at least 70 days, while 0.25% sodium diacetate or sodium acetate at 0.25 or 0.5% inhibited the pathogen growth for 20 to 50 days. Hence, according to this study, the tested organic salts could strongly be applied to control listerial postprocessing contamination on vacuum refrigerated frankfurters.

Furthermore, it has been reported that the microbial stability of refrigerated fresh ground pork meat was enhanced by the addition of 2% sodium or potassium lactates without deleterious effects on its colour or fat stability during 15 days of storage at 2°C [71].

Besides, sodium lactate at 1.8% and sodium diacetate at 0.25% displayed antilisterial effects in pork frankfurter samples without affecting the flavour and the overall acceptability of the meat products [72].

Moreover, it was reported by Carpenter et al. [62] that spray washing with 2% of lactic or acetic acids as decontamination solutions showed efficacy in reducing *Salmonella* populations on pork belly and in protecting meats against later growth of this pathogen.

In the study of Hwang et al. [73], it was shown that 1, 2, and 3% of lactate significantly ($P < 0.05$) inhibited the growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in cooked ham stored at refrigerated and abuse temperatures (4–15°C), and reduction effects were more pronounced at low storage temperatures. Therefore, it has been deduced that these lactate concentrations (1–3%) could be

eventually used to enhance microbiological safety of ready-to-eat ham products.

Added to that, it was demonstrated that potassium lactate at 2% inhibited the growth of *L. monocytogenes* and *Salmonella* spp. in cured pork meats for 49 days of refrigerated storage [64].

A recent study [74] on the residual antimicrobial effect of weak organic acids (lactic and acetic acids at 3%) on spoilage psychrotrophic germs in pig carcasses reported that the spraying decontamination of porcine carcasses with 3% of lactic or acetic acids resulted in an obvious reductions of psychrotrophs, *Pseudomonas* spp., and Enterobacteriaceae germs and the most sensitive bacteria were *Aeromonas* spp. and *Yersinia* spp. which were completely inhibited after 24 h of acid treatment.

Acetic, lactic, and citric acids at concentrations of 1, 2, or 3% were also assessed for their antimicrobial effects against *S.* Enteritidis, *L. monocytogenes*, *C. jejuni*, *E. coli*, and *Y. enterocolitica* bacteria in the decontamination operation of meat pork [75]. Analyses revealed that all of the tested organic acids could diminish the pathogen counts with great reductions shown with the concentration of 3%. The most efficient organic acid was lactic acid, followed by acetic acid and then citric acid.

Finally, González Sánchez [76] evaluated the effectiveness of formic acid (1.5%) and peroxyacetic acid (400 ppm) as antimicrobial spray formulations to control *Salmonella enterica* growth in chilled pork jowls. Overall, the acid spray treatments were effective in reducing *Salmonella* counts immediately after application and after 24 h of refrigerated storage at 4°C.

3.2.3. Sheep Meat. Decontamination of sheep carcasses by spraying lactic acid at 2% after slaughter and one-day cold storage resulted in significant ($P < 0.05$) reduction rates of total viable bacteria, coliforms, and *E. coli*, suggesting that the application of lactic acid at 2% with proper hygiene and handling procedures could provide safer sheep meat [77].

Recently, it has been found that sodium lactate marinating at 2% improved the water-holding capacity, solubility of the protein fraction, and marinade uptake of sheep meat [78].

Moreover, the addition of citric acid in sheep "Buchada" resulted in the reduction of microbial growth (thermotolerant coliforms, *Staphylococcus* spp., and *Salmonella* sp.), pH, moisture, and TBARS value and did not negatively affect the sensory attributes (odour, flavour, appearance, and tenderness) [57].

Finally, the antibacterial effect of lactic and acetic acid spray treatments, at 1, 1.5, and 2%, was studied towards the aerobic plate count, Enterobacteriaceae, coliform, and *Staphylococcus* counts on fresh sheep carcasses surfaces. The findings showed significant reductions in the microbial growth of the investigated microorganisms after sheep meat exposition to organic acids with specifically higher effect of 2% lactic acid spraying wash [79].

3.2.4. Poultry Meat Products. In chicken meat vacuum-infused with acetic, citric, lactic, malic, and tartaric acids (each at 75.0 or 150.0 mM) over 12 days of storage at 4°C, the *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella typhimurium* populations were significantly ($P < 0.05$) reduced by 1–6 log CFU/g, and 150.0 mM was the most effective antimicrobial concentration [80]. In addition, the effects of lactic acid at 0.2, 0.3, 0.5, 0.6, 0.8, and 1% and its sodium lactate salt at 1, 1.5, 2, 2.5, and 3% were assessed on chemical, microbiological, and sensory properties of marinated chicken thighs stored at 4°C for 15 days [81]. The results revealed that these additives were efficient against the proliferation of different spoilage and pathogenic microorganisms such as aerobic and psychrotrophic bacteria, *Pseudomonas* spp., *Salmonella* spp., Enterobacteriaceae, and *Staphylococcus aureus*. Chemical assessment indicated that the tested organic acid and its salt generally reduced the pH value (≈6.06 at the end of the storage period) and the total volatile basic nitrogen contents (TVBN) (with 0.2, 0.5, and 1% of lactic acid, and 1, 2, and 3% of sodium lactate) in treated chicken samples. Furthermore, lactic acid and sodium lactate improved the sensory attributes (odour, flavour, colour, and texture) with highest overall acceptability score (7.94 out of 10-point hedonic scale note) observed with 1% lactic acid followed by 0.8% lactic acid (score of 7.38) and 3% sodium lactate (score of 6.94). All of these results allowed a significant prolongation of the shelf-life of the refrigerated chicken products with the general order of the used organic preservatives as lactic acid > sodium lactate.

Another study in this context was previously conducted by Carpenter et al. [62] to evaluate the efficacy of washing chicken skin and turkey roll surfaces with solutions formulated with acetic or lactic acid at 2% for *Salmonella* and *L. monocytogenes* decontaminations, respectively [62]. The results revealed that lactic acid was the best organic agent in lowering the numbers of *Salmonella* on chicken skin and *L. monocytogenes* on turkey roll. On the other hand, acetic acid provided effective residual protection against later growth of these pathogenic bacteria than that displayed by lactic acid.

Moreover, it was shown that 2% potassium lactate strongly reduce *L. monocytogenes* and *Salmonella* spp. counts in refrigerated chicken Bologna samples, thus maintaining their microbial stability and shelf-life until 49 days of chilled storage [64].

Then, it was demonstrated that lactic acid (1 or 2%) inhibited the microbial growth of aerobic mesophilic, psychrotrophic, coliforms, and faecal coliforms bacteria as well as *E. coli* and *S. aureus* in broiler chicken breasts at 3°C [82]. Also, this organic acid could significantly ($P < 0.05$) reduce the TBARS and TVBN values compared to control samples, and the most pronounced reductions were provided by 2% lactic acid.

İlhak et al. [83] evaluated the effects of lactic acid or sodium lactate at 2 or 4% on psychrophilic bacteria and *Salmonella* spp. on chicken drumstick meats. In fact, spraying 4% lactic acid or 4% sodium lactate was most efficient than that applied with 2% concentration. In addition, the data showed immediate significant reductions of the *Salmonella* spp. counts by 1.3 and 1.1 $\log_{10}$ CFU/ml, respectively, and psychrophilic bacteria by 1.8 and 1.3 $\log_{10}$ CFU/ml, respectively, on chicken samples.

The application of lactic acid at 1% demonstrated interesting preservative effects on the quality of refrigerated chicken broiler fillets in terms of freshness (significant

decrease of TVBN and TBARS values), great control of microbial parameters (reduction of total psychrotrophic bacteria and Enterobacteriaceae loads), and inhibition of biogenic amine formation which increase the acceptability of refrigerated chicken meat up to 12 and 15 days at 4°C [84].

Findings of a recent study [76] on the decontamination efficacy of formic acid (1.5%) and peroxyacetic acid (550 ppm) against *C. jejuni* growth in chicken wings stored at 4°C for 24 h by immersion and spraying indicated that acid treatments were effective in decreasing the *C. jejuni* counts regardless of the antimicrobial application method.

Therefore, considering all of the abovementioned applications of organic acids (lactic, citric, acetic, formic, malic, and tartaric acids) and their salts as shelf-life extenders and bioagents in the preservation of different meat matrices during refrigerated storage, it has been indicated that mostly the weak organic acids and their salts were commonly used in such purposes. In fact, these latter are the most well-studied agents, more effective than other organic acids/salts, more available and naturally abundant, and finally legislatively allowed to be applied with *quantum satis* which favour their use in meat industry to replace the commonly used and expensive chemical preservatives, ensure their safety, improve their quality, and safely extend their shelf-life. Table 5 summarises some research results found around the globe about applications of organic acids and their salts for the preservation of various meat systems.

4. Application Types

Organic acids and their salt application in several meat models to control the microbial growth could be realised using different methods: spraying, dipping, injection, and incorporation into bioactive packaging films.

4.1. Spraying, Dipping, and Injection. Spraying of meat products with organic acid/salt solutions was found to offer significant protection against several bacteria and fungi. This application method is usually used in the early steps of meat carcass processing: after hide removal and before or after evisceration, but before the carcasses are chilled [3]. Moreover, organic acid spray is typically applied as a rinse to the entire carcass surface. The most common types of organic acid spray solutions widely available for carcass decontamination rinses are acetic and lactic acids, as well as sodium lactate [66, 74, 79, 83]. To ensure the success of the antimicrobial rinse, several steps should be performed carefully. Firstly, the carcass should be washed from top to bottom with warm water, followed by a five-minute dip. Then the organic acid/salt solution should be applied from top to bottom in two passes with a spray nozzle within 12 inches of the carcass surface, using a gentle sweeping motion, to cover the carcass completely (organic acid dip is a sign that the surface is saturated with organic solution).

Organic acid dipping represents another application type aiming to preserve microbial and sensory qualities of different meat matrices. It consists of immersion of the meat samples into organic acid/salt solutions at adequate concentrations for a time period (in minutes) [76]. Of the organic acids/salts evaluated in the literature, lactic, citric, and malic acids have been the most widely accepted as antimicrobial dip solution [85].

On the other hand, solutions of organic acids/salts could be injected into meat using a needle and syringe to improve its microbial, physicochemical, and sensory quality properties [86].

4.2. Incorporation of Organic Acids/Salts in Bioactive Packaging Films. Innovative concept of antimicrobial packaging has been introduced as a response to market trends and consumer desire. It consists of an active packaging where the antimicrobial agents are incorporated into the package material and from there migrate into the food through diffusion and partitioning. Direct incorporation of organic acids/salts as preservatives into bioactive packaging (by extrusion, compression moulding, casting, or coating) is an advanced technology responding to many problems and an economical method for manufacturing antimicrobial packaging. Effectively, they were proposed to overcome the loss of organic acid/salt activity when directly applied into meat samples due to the interaction with meat components (e.g., fat, skin, and enzymes). These techniques depend on the release of such acidic agents from the packaging to the meat matrix, thus avoiding direct contact with the meat product which responds to the consumer desire of having safe meats free of preservatives or with few or natural ones. However, it is important to keep in mind that there is always a dose-effect relation, so preservative agents should be applied in sufficient amounts, to obtain high effect, which is furthermore dependent on the meat properties [6]. Furthermore, the thickness of the material and the storage conditions in terms of temperature and time should be properly taken into account as important factors on which depend the antimicrobial/preservative actions of the active packaging containing organic acids/salts.

Other possible methods for the application of organic acids include the use of edible antimicrobial films: this method has received attention as a potential intervention strategy against pathogens in various meat muscle samples [3]. These antimicrobial films were prepared by dissolving chitosan into hydrochloric acid and organic acid solutions such as formic, acetic, lactic, and citric acid solutions.

5. Combinations between Organic Acids/Their Salts and Other Antimicrobials or Preservation Methods

The main objective of the combinations between organic acids/salts with other control measures (chemical, physical, and biological treatments) is to obtain potent synergistic activities in terms of antimicrobial effect enhancement, sensory quality improvement, and meat shelf-life extending which is quite beneficial to meat industries.

5.1. Combination of Organic Acids/Their Salts with Chemicals. The most common practice previously applied in this context is the addition of sodium chloride (NaCl) salt with

Table 5: Applications of organic acid and their salts in meat preservation.

Meat products	Organic acid/salt	Concentration	Lipid and protein oxidation inhibition activity	Effective antimicrobial activity	Sensory attributes	Shelf-life extension	Reference
Raw beef during vacuum-packaged storage at 2°C for 21 days	Sodium lactate	3 g/kg	TBARS value not affected	Reduction of aerobic plate counts, psychrotrophic counts, LAB, Enterobacteriaceae	—	Up to 15 days against 8 days for control	[59]
Beef trimmings	Potassium lactate	3%	TBARS value not affected	—	Improvement of odour, taste, colour, tenderness, and cooking yield	—	[60]
Roast beef	Potassium lactate	2%	—	Decrease of *L. monocytogenes*, *Salmonella* spp.	—	—	[64]
Raw beef meat stored at 4°C	Lactic acid Acetic acid Sodium lactate Sodium acetate	1–2% 1–2% 2.5% 2.5%	—	Reduction of aerobic plate and psychrotrophic counts, Enterobacteriaceae	Improvement of colour, flavour, texture	Significant extension of shelf-life	[63]
Fresh ground pork stored at 2°C and −20°C	Sodium or potassium lactates	2%	TBARS value not affected	Enhancement of microbial stability	No negative effect on colour	15 days (at 2°C) and up to 70 days (at −20°C)	[71]
Pork frankfurters	Sodium lactate Sodium diacetate	1.8% 0.25%	—	Decrease of *L. monocytogenes*	No negative effect on flavour	—	[72]
Porcine carcasses	Lactic acid Acetic acid	3% 3%	—	Decline of psychrotrophs, *Pseudomonas* spp., Enterobacteriaceae, *Aeromonas* spp., *Yersinia* spp.	—	—	[75]
Chilled pork jowls	Formic acid	1.5%	—	Reduction of *Salmonella enterica*	—	—	[76]
Sheep "Buchada" meat	Citric acid	1%	Reduction of TBARS value	Reduction of *Salmonella* spp., Staphylococcus spp., and thermotolerant coliforms	No negative effect on odour, flavour, appearance, and tenderness	—	[57]
Marinated chicken thighs stored at 4°C for 15 days	Lactic acid Sodium lactate	0.2–1% 1–3%	Reduction of TVBN contents	Reduction of aerobic and psychrophilic bacteria, Enterobacteriaceae, *Salmonella* spp., *S. aureus*, and *Pseudomonas* spp.	Improvement of odour, flavour, colour, and texture	Significant extension of shelf-life	[81]
Refrigerated chicken Bologna	Potassium lactate	2%	—	Decrease of *L. monocytogenes* and *Salmonella* spp.	—	Until 49 days	[64]
Broiler chicken breasts chilled at 3°C	Lactic acid	1 or 2%	Reduction of TVBN and TBARS values	Reduction of aerobic mesophilic bacteria, psychrotrophic counts, coliforms and faecal coliforms, *E. coli,* and *S. aureus*	—	Significant extension of shelf-life	[82]
Chicken broiler fillets refrigerated at 4°C	Lactic acid	1%	Decrease of TVBN and TBARS values	Decrease of total psychrotrophic bacteria and Enterobacteriaceae	Inhibition of biogenic amine formation	Up to 12 and 15 days	[84]

some organic acids or organic acid salts [3]. Effectively, Tan and Shelef [71] indicated that the combinations of sodium lactate or potassium lactate at 2% with NaCl at 1 or 2% were more effective than lactates alone at 2% in inhibiting the growth of spoilage microorganisms on refrigerated fresh ground pork meat. In addition, these combinations improved the fat stability by reducing the prooxidant effects of NaCl and significantly enhanced the red colour of meat after their immediate addition.

Sallam and Samejima [59] reported that the combination (20 + 20 g/kg) of sodium lactate and NaCl on raw ground beef could be successfully used to reduce the growth of aerobic plate, psychrotrophic, LAB, and Enterobacteriaceae counts, maintain its physicochemical quality by reducing the oxidative changes caused by NaCl, and considerably prolong its shelf-life during refrigerated storage to 21 days compared to control (only 8 days).

Furthermore, it was reported by Entani et al. [87] that the application of acetic acid in the form of vinegar with NaCl resulted in a great synergistic effect towards the inhibition of *E. coli* O157:H7. This antimicrobial activity was explained by the fact that NaCl induced *E. coli* O157:H7 sensitivity to the acid environment. Indeed, it was stated by Casey and Condon [88] that NaCl decreases the antimicrobial activity of lactic acid on this tested pathogen by increasing its cytoplasmic pH. Hence, the presence of NaCl protected *E. coli* O157:H45 against the inhibitory effect of acid pH. This aspect seems to be of considerable importance in food safety as it falls in the abovementioned resistance mechanisms of some microorganisms (such as the case of *E. coli* O157:H45) to counteract acidification, escape to the bactericidal effect of the used organic acid, and cause illnesses by surviving in the food product.

Otherwise, some other preservation methods that are safer and more effective could be proposed to meat industry. In fact, it was indicated by Barmpalia et al. [89] that the combination of 1.8% sodium lactate + 0.125% sodium diacetate + 0.125% glucono-delta-lactone (GDL) significantly inhibited the growth of *L. monocytogenes* and spoilage LAB in pork Bologna meats stored at 4 or at 10°C as a mildly abusive storage temperature. Furthermore, Jo et al. [90] reported that combined treatments of silver ion (at 0.1 or 0.3 or 0.5 or 1 ppm) with (0.05% or 0.1%) organic acid (lactic or citric or succinic or maleic or tartaric) enhanced the inhibitory effect of *E. coli* O157:H7 and the more effective combination was 1 ppm silver ion +0.1% organic acid.

5.2. Combinations of Organic Acids Together, or Organic Salts Together or Organic Acids with Organic Salts. The use of mixtures containing different organic acids together or formulations with several organic acid salts together or combinations of organic acids with their organic salts could also be useful technological strategies in enhancing not only the antimicrobial activity but also preservative effect on the treated meat product.

For instance, the combinations of 2.5% sodium lactate +0.3% diacetate and 2.5% sodium lactate +0.1% diacetate provided significant antilisterial activity on turkey slurries stored at 25°C and 4°C, respectively, compared to treatments containing sodium lactate or diacetate alone [91].

Then, it was reported by Mbandi and Shelef [92] that synergistic effect between sodium lactate and sodium diacetate on microbial growth occurred in beef meat. Indeed, the combination of sodium lactate and sodium diacetate, respectively, at 2.5 and 2%, was bacteriostatic to *L. monocytogenes* and bactericidal to *Salmonella* Enteritidis after 20 days of beef storage at 10°C. On the other hand, 1.8% sodium lactate +0.1% sodium diacetate provided a listeriostatic effect, while the *Salmonella* counts were less than 10 cells/g after 30 days of refrigerated storage at 5°C. In addition, antimicrobial activity against both tested pathogens was strongly enhanced by the application of 0.2% sodium acetate in combination with 1.8 or 2.5 % sodium lactate.

Later, Mbandi and Shelef [93] stated similar results regarding the rapid and efficient decrease of *Salmonella* counts in beef Bologna meat when lactate and diacetate were combined and applied as meat preservative formulation.

Furthermore, it has been demonstrated that a spray washing method with 2% lactic acid + 1.5% propionic acid or 1.5% acetic acid + 1.5% propionic acid combinations remarkably declined total viable counts to undetectable levels during storage of fresh sheep/goat meats compared to the single acid treatments [94]. Added to that, the shelf-life of meat samples treated with the binary organic acids combinations was increased to 11 days *versus* 3 days in untreated meats.

On the other hand, in a study undertaken by Juneja and Thippareddi [95], it was indicated that the combinations of sodium diacetate with sodium lactate, or sodium acetate or buffered sodium citrate significantly inhibit the germination and outgrowth of *C. perfringens* from spores in processed chilled turkey products.

Barmpalia et al. [72] evaluated the control of *L. monocytogenes* on refrigerated pork frankfurters by applying a combination containing 1.8% sodium lactate and 0.125% or 0.25% sodium diacetate followed or not by a dipping in 2.5% lactic acid or acetic acid solutions. The findings revealed that the combinations with acid dipping resulted in marked reductions of *L. monocytogenes*, LAB, yeasts, and moulds populations, with highest antimicrobial effects (complete inhibition) shown with 0.25% sodium diacetate over 12 days. In addition, using sodium lactate and sodium diacetate in combination, with or without organic acid dipping, did not affect the flavour and the overall acceptability of pork products compared to controls.

Similarly, Barmpalia et al. [89] indicated that 1.8% sodium lactate + 0.25% sodium diacetate combination controlled the proliferation of *L. monocytogenes* and spoilage LAB in refrigerated pork Bologna more effectively than the treatments when the organic acid salts were used as single antimicrobial agent.

Drosinos et al. [96] highlighted enhanced inhibitory activity against spoilage flora in both culture medium and cured cooked meat products when two or three organic acid salts were combined together as follows: 2% sodium lactate + 0.5% sodium acetate, 2% sodium lactate + 0.15% potassium sorbate, and 2–4% sodium lactate + 0.5% sodium acetate + 0.15% potassium sorbate.

Likewise, the combination of citric, malic, and tartaric acids at 150 mM strongly reduced the growth of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella typhimurium* populations by $>5>2$ and 4–6 log CFU/g and significantly extended the shelf-life of chicken breast pieces [80].

In addition, Samoui et al. [81] found that the combination of 0.9% sodium lactate and 0.09% lactic acid is the most effective in delaying the proliferation of spoilage bacteria (aerobic plate counts, psychrotrophic populations, Enterobacteriaceae, *Pseudomonas* spp., *S. aureus*, and *Salmonella* spp.), preventing the generation of undesirable chemicals (TVBN), improving the sensory quality of chicken meats (highest scores for the colour, flavour, and texture), and extending the shelf-life during chilled storage.

Recently, it has been found that the combinations of 1.5% acetic acid + 400 ppm peroxyacetic acid and 1.5% formic acid + 400 ppm peroxyacetic acid were highly effective in declining the *Salmonella* counts on chicken wings, while *C. jejuni* were mostly sensitive to the combination containing 1.5% formic acid + 550 ppm peroxyacetic acid [76].

5.3. Combinations of Organic Acids/Their Salts with Bacteriocins. Schlyter et al. [91] reported that diacetate at 0.3 or 0.5% combined with pediocin (5000 AU/ml) provided a listericidal effect on turkey slurries at 4°C and 25°C, respectively.

Acetic acid at 3 or 5 g/100 ml, or sodium diacetate at 3 or 5 g/100 ml, or potassium benzoate at 3 g/100 ml combined with nisin (5000 IU/ml) as dipping solutions have been described to cause an important reduction of *L. monocytogenes* populations in sliced cured pork Bologna meats with a significant increase of their shelf-life for 90–120 days [32].

Khalafalla et al. [82] studied the combination of lactic acid (1%) with nisin at 50 μg or 100 μg strongly improved the microbial (aerobic mesophilic, aerobic psychrophilic, coliforms, faecal coliforms, *E. coli*, and *S. aureus* counts) and physicochemical (pH, TBARS) qualities of chicken breast samples and significantly extended their shelf-life to 12 days during refrigerated storage at 3°C.

5.4. Combinations of Organic Acids/Their Salts with Essential Oils or Aromatic/Phenolic Compounds Obtained from Plants. In this line, de Souza et al. [97] reported that the combined application of *Origanum vulgare* L. essential oil (MIC × ½: 0.3 μl/ml) and acetic acid (MIC × ½: 0.3 μl/ml) inhibited the growth of *S. aureus* populations on bovine meat steaks.

İlhak et al. [83] proposed an effective technological concept against the proliferation of undesirable bacteria in refrigerated chicken drumstick samples consisting of the combination between lactic acid (4%) + thymol (0.25%, w/v) applied by spraying method. The results showed a significant and immediate decline of *Salmonella* spp. and psychrophilic numbers by 1.4 and 1.8 $\log_{10}$ CFU/ml on day 0, respectively, with an interesting pH stability of the meats during the refrigerated storage at 4°C as compared to treatments with lactic acid or thymol alone. Indeed, the synergistic antimicrobial effect of these two antimicrobials could be explained by the fact that the phenolic compound thymol could contribute to facilitating the diffusion of lactic acid into the microorganism's cytoplasm through disintegration of its outer membrane, thus increasing the permeability of the cytoplasmic membrane [98]. However, the use of thymol (or other aromatic compounds such as eugenol, carvacrol, geraniol, and citral) at higher concentrations, when they are applied individually for sufficient antimicrobial activity, could negatively affect the meat flavour, thus being unacceptable to consumers. That is why combinations of essential oils with other preservative agents in meats (such as organic acids/salts) have been applied to strongly minimise the application concentrations required.

Recent research by Gómez-García et al. [16] reported similar results concerning the enhancement of antimicrobial effects towards swine *Salmonella* spp. for formic acid-carvacrol and formic acid-thymol binary combinations.

Finally, it is quite important to mention that the combination of more than two antimicrobials is also possible to achieve preservative efficacy of tested meat systems by either synergistic or additive effects and therefore ensure both safety and quality of meat products. In this context, Ghabraie et al. [99] tested the antilisterial effects of antibacterial formulations containing organic acid salts, essential oils, nisin, and nitrite combined at different concentrations in fresh beef and fresh pork sausages. The results demonstrated that the formulation A (mixed organic acid salts: sodium acetate and potassium lactate at 1.55% (w/w)) + mixed Chinese cinnamon and cinnamon bark essential oils (EO) at 0.05% (v/w) + nisin at 12.5 ppm + nitrite at 100 ppm) and formulation B (mixed organic acid salts (1.55%, w/w) + mixed EOs (0.025%, v/w) + nisin (12.5 ppm) + nitrite (100 ppm)) remarkably ($P < 0.05$) reduced *L. monocytogenes* numbers. The two formulations (A and B) were also organoleptically (texture, smell, and taste) accepted in both fresh pork and beef sausages.

5.5. Combinations of Organic Acids/Their Salts with Plant Extracts. The antimicrobial effectiveness of the combination between tartaric acid (37.5 mM) and grape seed (GS) or green tea (GT) extracts (at 5 or 10 or 20 or 40 mg/ml) was studied by Over et al. [80]. The findings revealed that all binary combinations strongly decreased the numbers of *Salmonella typhimurium*, *L. monocytogenes*, and *E. coli* O157:H7. However, these high antibacterial activities of tartaric acid combined with GS or GT were observed in BHI broths, and it was suggested to assess the effect of the highest antimicrobial combination on microbial, physicochemical, and organoleptic properties of poultry meat system (chicken breasts).

The study of Kim et al. [100] was conducted to improve the quality of cured pork loin with a combination of an organic acid (ascorbic, malic, citric, or tartaric acids) at 0.06% and fermented spinach (FS) as green nitrite source at 0.08%. The results showed that cured pork meats treated with fermented spinach (natural nitrite) with each organic acid possessed higher redness values than control on cooked

meat. In addition, all combinations significantly reduced the lipid oxidation (TBARS and TVBN) except the citric acid when combined to FS. Among the combinations, ascorbic acid + FS was found to provide the highest efficacy on quality attributes of cured pork meat samples.

5.6. Combinations of Organic Acids/Their Salts with Bacteriophages. Wang et al. [34] stated in their recent study that the combination of potassium sorbate (2 mg/g) + nisin (5000 IU/g) + bacteriophage (9 log PFU) significantly reduced *Salmonella* counts, total viable counts, TVBN, and TBARS on fresh chilled pork. In addition, this combination strongly reduced odour and maintained good organoleptic properties of fresh pork meat, thus extending its shelf-life up to 14 days. It has been shown too that no adverse effect of the phage on meat samples was observed.

Otherwise, Shebs et al. [101] evaluated the effect of the combination between peroxyacetic acid (PAA, 400 ppm) and bacteriophage (P, 7 MS phages at 10^8 PFU/ml) against Shiga toxin-producing *E. coli* (STEC) (O157:H7 and O145, O121, O111, O103, O45, and O26) on beef meat. The findings showed that this combination led to a significant STEC reduction by 1.49 log compared to control and treatment with PAA alone.

5.7. Combinations of Organic Acids/Their Salts with High Pressure Processing (HPP). Similarly, Rodríguez-Calleja et al. [102] have demonstrated the synergetic effect of a mix of organic acids (lactic and acetic acids + sodium diacetate) and HPP (300 MPa) in extending the shelf-life of skinless chicken breast fillets up to 28 days. A remarkable maintain of the microbiological quality (total viable counts, LAB, coliforms, *E. coli*, *Pseudomonas*, and *Brochothrix thermosphacta*) and sensory properties (colour, tenderness, and overall acceptability) was reported.

Recently, O′ Neill et al. [103] evaluated the effectiveness of a combination between 0.3% of the commercial mix of organic acids named Inbac^{TM} (mainly composed of 43% sodium acetate and 7% malic acid and other fatty acids and technological coadjuvants) and high HPP as preservative technique to extend the shelf-life of frankfurters and cooked ham. As a result, it has been observed that the combinations of HPP at 580 MPa or 535 MPa for 5 min and 0.3% Inbac^{TM} organic acid mixture significantly decrease the total aerobic viable counts, total coliforms, *E. coli*, and *Salmonella* spp. in frankfurters and cooked ham, respectively. Hence, these combinations (organic acids + HPP) greatly extended the shelf-life of frankfurters by 51% and cooked ham by 97% compared to control samples from the microbiological point of view; however, no significant improvement effects were detected for physicochemical and sensory qualities in terms of pH and TBARS values, colour, flavour, off-flavour intensity, texture, juiciness, tenderness, saltiness, and overall acceptability.

5.8. Combinations of Organic Acids/Their Salts with Heat or Ultraviolet Light (UV). Some interventions such as pasteurization with steam or hot water combined with organic acid solutions were among the advanced processes to improve the meat safety that resulted in more effective reductions of spoilage and pathogenic microorganisms on meat carcasses or meat end-products [104].

On the other hand, it was reported that, under vacuum and aerobic conditions, the application of peroxyacetic acid (PAA, 400 ppm) in combination with ultraviolet light (UV, 30 s at 2.5 ± 0.3 cm height) provided high STEC reductions on beef meat samples compared to the results obtained with samples treated only with 400 ppm PAA [101].

5.9. Combinations of Organic Acids/Their Salts with Modified Atmosphere Packaging (MAP) or Bioactive Packaging Films. Regarding MAP preservation technique, it has been shown previously that application of 1% acetic acid solution combined with MAP in a 70% CO_2/30% N_2 on chicken breasts portions reduced microbial growth and maintained pleasant flavour at the end of the storage period (21 days at 4°C) [105].

Likewise, the microbial stability (total viable counts, LAB, coliforms, *E. coli*, *Pseudomonas*, and *Brochothrix thermosphacta*) of fresh chicken breast fillets was significantly enhanced by the combination between a commercial organic acid mixture composed principally of lactic acid, acetic acid, and sodium acetate, and MAP (30% CO_2/70% N_2) [102]. Hence, a prolongation of shelf-life was observed to be two weeks *versus* one week for untreated chicken fillets. Besides, in this same study, it was shown that the organic acids + MAP (30% CO_2/70% N_2) + HPP (300 MPa) combination was the most efficient preservative strategy in inhibiting spoilage bacteria, maintaining organoleptic attributes in high scores, and extending the shelf-life of chicken samples.

Otherwise, organic acids/salts could be incorporated, alone or in combination, in bioactive packaging (by extrusion, compression moulding, casting, or coating) as advanced antimicrobial application in meat industry. According to the published literature, the incorporation of organic acids/salts during the extrusion or plastic compressing moulding is the most economical technique since it did not require additional processing steps [6]. However, the applicability of this approach is not feasible with all organic acids/salts as some of them are characterised by a liquid state at room temperature or by heat sensitivity. Hence, it has been indicated that sorbic acid and potassium sorbate are the commonly used agents into such packaging. Occasionally, some issues could be encountered regarding the direct incorporation of organic acid/salts into packaging. For instance, incompatibility between the organic acid/salt and some biopolymers (low-density polyethylene (LDPE) or linear low-density polyethylene (LLDPE)) could be happened leading to the loss of antimicrobial effect [106], or causing loss of transparency of biofilms after extrusion [6]. For this reason, their indirect incorporation by casting or coating was suggested.

Regarding the inclusion of organic acids/slats into packaging films by casting, nonpolar polymers (e.g., polyethylene and cellulose polymers) and polar polymers (starch, alginate, chitosan, and carrageenan) could be used in the

process [6]. In fact, Limjaroen et al. [33] reported a marked *L. monocytogenes* inhibition for up to 28 days in refrigerated beef Bologna sausages packed with polyvinylidene chloride (nonpolar) films containing 1.5% sorbic acid. Furthermore, Zinoviadou et al. [107] indicated that total viable counts and *Pseudomonas* spp. populations were significantly reduced on fresh beef cut portions stored at 5°C for 12 days in whey protein (polar) films containing 2% sodium lactate. Similarly, da Rocha et al. [29] showed that protein-based films containing 1.5% sorbic or benzoic acid are strongly efficient to decline *E. coli* and *L. monocytogenes* counts by 5–6 log-cycles on meat samples stored at 5°C for 12 days.

Concerning the incorporation of organic acids/slats into packaging films by coating, it has been shown by Hauser and Wunderlich [108] that sorbic acid coating incorporated in polyvinyl acetate lacquer on LDPE films strongly decreased *E. coli* counts in pork loin meats stored at 8°C up to 7 days.

On the other hand, it could be important to mention the existence of other rare techniques used for incorporating organic acids into polymer films such as the immersion of polyamide films into solution of lactic acid followed by a drying step as performed by Smulders et al. [109]. These films were effective in inhibiting the growth of *E. coli* O157: H7 on refrigerated fresh beef cuts by 1 log-cycle, reaching a final 2 log reduction after 14 days.

Therefore, all of these organic acid-based antimicrobial packaging systems seem to be low-cost technologies and easy to apply which makes them a promising additional approach useful in meat preservation. Moreover, the use of such active packaging is regulated in Europe by the Regulation (EC) No. 450/2009 on Active and Intelligent Materials [110] and the organic acids/salts used to be incorporated into antimicrobial packaging films for release into food (such as meat) have to be listed as food preservative agents in the Regulation (EC) No. 1333/2008 on Food Additives which also regulates their migration limits [8]. Finally, concerning the labelling of antimicrobial additives (such as organic acids/salts) in food packaging materials, the Regulation (EC) No. 450/2009 (article 11) declares that every released active substance is imperatively considered as a food ingredient and must be declared accordingly [110].

6. Limits of Organic Acids and Their Salts Use in Meat Biopreservation

The main purpose of organic acids and their salts use as preservative agents in meat and meat products was to control the growth of spoilage and pathogenic bacteria and extend the shelf-life of meat products without causing sensory changes. However, it is well known that the concentrations of organic acids/salts applied to obtain efficient antimicrobial activity in meats should be higher than those used in laboratory media. Hence, this increase in concentrations could dramatically change the sensory quality (colour, odour, flavour, and taste) of meat samples [19].

Otherwise, the insufficiency or lack of antimicrobial/preservative effect of organic acids and their salts added to meat and meat products could depend not only on the concentration parameter but also on possible interferences between the meat matrix, via its intrinsic parameters (nonhomogeneous, moisture content, pH relatively high, high content of protein and fat, and quantity and types of occurred microorganisms), and the used acid antimicrobials which represent a further concern associated with the application of organic acids and their salts in meat.

Another limit that could also be mentioned here is the incompatibility between organic acids/salts and other inoculated antimicrobial or preservative agents in meats. For this reason, strict assessments of this aspect should be carefully performed before such applications. In addition to this, despite the fact that organic acids have been proven for many years to be effective as biopreservatives in meat industry, there is increasing indication that some microorganisms possess various mechanisms to counteract the effects of such acid agents, thus allowing for resistance to antimicrobial activity and/or to severe acidic conditions [3, 6]. Therefore, it has been noted that this fact resulted in the emergence of acid-tolerant food-borne pathogens [111]. In fact, resistance mechanisms are known to be stress mechanisms at the cellular activity level implicating, for example, the expression of specific stress-response genes. For instance, *E. coli*, *Salmonella typhimurium*, and *L. monocytogenes* have the possibility to develop cells inducing acid tolerance response (ATR) mechanisms at low pH levels after preliminary adaptation at moderately acidified conditions [6]. These acid-adapted cells are more resistant and so more infective under stress conditions. Other acid-tolerant microorganisms developed cross-resistance to other environmental stresses such as salt, osmosis, and heat [3]. On the other hand, some other microorganisms (e.g., *P. aeruginosa*, *Aspergillus* spp., and *Penicillium roqueforti*) exhibit different counteracting mechanisms towards organic acids/salts which could be degraded by some bacterial or fungal enzymes [3, 6, 112]. These enzymatic degradations could thus unfavourably affect the organoleptic characteristics of meats. Otherwise, the yeast species of *Zygosaccharomyces bailii*, which is known to be more resistant to organic acids than *S. cerevisiae*, was able to reduce the diffusion of weak organic acids across the membrane by adjusting its cell envelop confirmation [113, 114].

Furthermore, although there are wide numbers of potent organic acids and salts newly characterised for their effectiveness as powerful natural preservatives (other than the most frequently used ones in meat industry until now), relatively few ones are suitable in practice and so are legally approved as preservatives in meats by international food legislations to become commercially used. This could be mainly explained by the strict requirements taken from regulatory agencies prior to final approval of possible use of an organic acid/salt in meat products. Effectively, this process implicates detailed data regarding the functionality, the application method, and the safety aspects of the proposed organic acid/salt. Unfortunately, this approval procedure is very time-consuming because of its complexity in collecting all of these informative data after performing numerous *in vitro* and *in vivo* studies that could take many years and could also be expensive which limits the use of new organic acids/salts in meat biopreservation.

Besides, it is important to mention in this context that food legislation continued to restrict both changes in allowed organic acid/salt levels in meats even after completing rigorous studies and the use of some newly accepted ones as preservatives. For instance, despite regulatory approval, some organic acids/salts are not widely accepted in commercial practice particularly in meat decontamination. In fact, meat hygiene regulations within the European Union (EU) do not permit any method of decontaminated formulations other than washing meat carcasses with potable water [3]. A very good example of this is the peroxyacetic acid (also known as peracetic acid or PAA) which is approved in the United States and Australia and prohibited in the EU [115].

Moreover, despite the GRAS status of the organic acids/salts legislatively approved as food preservative agents, their innocuousness still raises questions regarding undesirable toxicological effects on human health after ingestion of meat products treated with these bioagents. In fact, according to the literature, some *in vitro* and *in vivo* studies on the toxicity of sorbic and benzoic acids and/or salts revealed no mutagenic, genotoxic, and/or carcinogenic effects, while some other researchers reported genotoxic effects of the two mentioned acids as well as their sodium and potassium salts [6]. This controversial safe status is not the case for lactic, citric, or propionic acids, maybe because they are natural intermediates of plants, animal, and human metabolisms, thus suggesting their harmlessness. Hence, this dual trait of being sometimes innocuous and sometimes toxic raises a great worry and a big issue regarding the use of organic acids/salts in meat preservation since a final elucidation about their toxicity is not yet established. Deep research is strongly recommended in this way.

Finally, it is quite important to notice that data on the effectiveness of organic acids and their salts applied in actual commercial practice for meat preservation are limited which are of glaring need to evaluate their continued efficiency basing on recent circumstances and to accurately improve their sustainability.

For these reasons, it is almost indisputable that, in some cases, the use of the commonly approved organic acids or their salts solely applied in meats could not result in sufficient or desired preservative effects. Consequently, to enhance their efficacy in meat preservation, their application in combination with other antimicrobials (organic acids, organic acid salts, proteins, bacteriocins, bacteriocinogenic LAB, plant extracts, essential oils, nanoparticles, bacteriophages, etc.), or chemicals (e.g., salts: NaCl) or diverse physical techniques (pressure, UV, hot water, pasteurization with steam, MAP, and bioactive packaging) is strongly recommended. These promising preservation strategies could open new pathways and opportunities for the successful use of efficient, safe, and cost-effective preservative formulations in meat preservation.

7. Conclusion

This review highlights the interesting attributes of organic acids and their salts as natural antimicrobials with high preservative effects in meat industry based on several conducted studies and published research, making them innovative bioagents instead of chemical preservatives. In fact, it has been demonstrated that the organic acids/salts are GRAS agents (when used in respect to the specified amounts) approved by EU legislation as preservatives (and other categorised technological agents: acidifiers, antioxidants, colour stabilisers, flavour enhancers, etc.) useful for meat applications (and other food matrices). These bioagents could strongly ensure the overall safety of meat products. They could inhibit or delay a wide range of spoilage and pathogenic microorganisms, improve the physicochemical properties of meats (TBARS, TVBN, and pH reductions), and improve their organoleptic attributes (stability of red colour, enhancement of flavour, suppression of rancidity and undesirable off-odour, inhibition of slime formation and gas production, improvement of tenderness and juiciness, etc.). In addition, it is important to keep in mind that the use of organic acids/salts as biopreservatives will not replace good manufacturing and hygienic practices and procedures.

However, despite all of these advantages, it has been shown, until now, that relatively few organic acids/salts are suitable in practice. Why? To answer this key question, many studies have been worldwide undertaken, and laboratory tests on food indicated the existence of possible problem areas regarding the effectiveness of organic acids/salts in meat biopreservation due to several factors implicating organic acid/salt characteristics (form, concentration, antimicrobial activity, etc.), meat (type, composition, microbial loads, pH, A_w, etc.), and environmental conditions (temperature, humidity, storage, packaging, etc.). In addition, extensive use of organic acids could result in bacterial adaptation, making some microorganisms acid-tolerant and resistant in some other ways, which represent challenges for their use as food preservatives, particularly in meats. This dilemma regarding the organic acids/salts dual trait of being sometimes good preservative agents and sometimes not suggests strict *in vitro* and *in vivo* assessments in terms of antimicrobial activity, preservative effects on physicochemical and sensory qualities, shelf-life prolongation, and safety aspects to accurately select efficient candidates, and much more importantly, well-adapted agents to each meat type where they will be applied. Moreover, it is crucial to achieve a better understanding of how pathogens or spoilage microorganisms respond to organic acids/salts when added to meat to develop optimal solutions and overcome related issues. Therefore, combinations of organic acids/salts with other control or preservative techniques (chemical, physical, and biological) were proposed to design improved preservation strategies based on potent synergistic activities or additional effects that could benefit the meat industries at cost level and meet the modern consumer trends for safe and "bio" meat products.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Erratum

Erratum to "Inhibitory Effects of *Litsea cubeba* Oil and Its Active Components on *Aspergillus flavus*"

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In the article titled "Inhibitory Effects of *Litsea cubeba* Oil and Its Active Components on *Aspergillus flavus*" [1], the author name Guorong Fan was omitted from the correspondence information in error. The correct correspondence line is "Correspondence should be addressed to Zongde Wang; zongdewang@163.com and Guorong Fan; fgr008@126.com". This mistake was introduced during the production process and Hindawi apologises for causing this error in the article.

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[1] S. Xia, H. Lin, P. Zhu et al., "Inhibitory Effects of *Litsea cubeba* Oil and its Active Components on *Aspergillus flavus*," *Journal of Food Quality*, vol. 2020, Article ID 8843251, 9 pages, 2020.

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Research Article

Effect of Pomegranate Juice on the Manufacturing Process and Characterization of Feta-Type Cheese during Storage

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The present research aimed to produce Fruit Feta-type cheese and investigate the effects of pomegranate juice on the manufacturing process and characterization of Feta-type cheese during storage. In order to produce Feta-type cheese with pomegranate juice, part of the milk was replaced with pomegranate juice. Therefore, it was necessary to produce Feta-type cheese with whey-less method, which is a good substitute for ultrafiltrated cheese. Initially, whey-less Feta-type cheese was produced. The formula was optimized based on the physicochemical characteristics of the marketed ultrafiltrated Feta cheeses. At the optimal point, the amount of cream, MPC, WPC, and fresh milk were 45.6, 11.7, 2.7, and 40%, respectively. Whey-less Feta-type cheese with these ratios was produced and the effects of different levels of pomegranate juice (0, 5, 10, 15, and 20%) on its physicochemical, textural, microbial, and sensorial properties during 60 days of storage were studied. The results showed that pomegranate juice increased acidity, total phenol, anthocyanin, DPPH inhibition, and a* value, and reduced the pH, peroxide value, thiobarbituric acid (TBA), proteolysis, lipolysis, and L* and b* values. Adding pomegranate juice to cheese reduced all of the textural indices except the adhesiveness. Sensory evaluation of the samples showed that the sample containing 20% of pomegranate juice had a higher score and was selected as the best sample.

1. Introduction

Cheese is one of the most consumed dairy products in most parts of the world. The diversity and the high nutritional value of cheese have given a special position for it in the diet of many countries. Feta is a semi-soft, white brined, protected designation of origin traditional Greek cheese, made from ovine milk or mixtures of it with caprine milk (up to 30%) [1]. Traditionally, Feta cheese is made from raw milk in small family premises with elementary equipment. Sometimes, the producers apply a milder than high-temperature short-time (HTST) heat treatment of milk and traditional yogurt used as a starter [2].Therefore, in order to increase the per capita consumption of cheese, new types of cheese with good nutritional and sensory properties should be produced.

In recent years, different types of fruit-flavored dairy products have been produced and marketed. However, there has been no specific study on the production of cheese containing fruit juice. In addition to the functional properties, this product can be accepted by consumers in terms of sensory and nutritional features. Dairy products, such as cheese, are able to carry functional compounds and release them in the body due to the ability to form a gel [3]. Adding these compounds to cheese can improve its antioxidant activity, functional properties, color, and nutritional value. There is some research regarding production of fruit cheese such as guava-based papaya fruit cheese [4], Gouda cheese supplemented with fruit liquors [5], functional cheese from goat milk supplemented with baobab fruit pulp [6], and guava cheese fortified with perishable loss of fruits [7].

Among fruits, pomegranate is rich in phenolic compounds and has antioxidant capacity and the potential to inhibit free radicals [8]. Pomegranate (*Punica granatum L.*) belongs to the Punicaceae family and is native from Iran to northern India. Numerous researches have shown that bioactive compounds in pomegranate juice are useful for lowering blood pressure, reducing blood cholesterol, preventing cardiovascular diseases, blocking blood vessels, and preventing a variety of cancers. The total pomegranate production in the world was estimated to be around 3 million tons in 2014 and 3.8 million tons in 2017. Due to the rapid increase in the production, it is highly difficult to calculate the total production [9]. Zarban et al. [8] studied the antioxidant activity and the ability to neutralize free radicals of nine commercial fruit juices, and observed that pomegranate juice had the highest phenolic compounds and total antioxidant activity. Han et al. [10] developed a functional cheese containing phenolic compounds and investigated the antioxidant properties of the samples. They used single-ring phenolic compounds, including catechin, tannic acid, and flavones, and natural compounds, such as grape extract, green tea extract, and corn powder, as functional compounds in the preparation of cheese. Cheese matrix containing 0.5 mg/mL polyphenolic compounds exhibited a significant effect in the absorption of free radicals.

In order to produce Feta-type cheese with pomegranate juice, it is necessary to replace part of the milk with pomegranate juice. The pH of pomegranate juice is low and it cannot be added directly to milk; therefore, milk protein powder should be added to fresh milk first and then pomegranate juice should be added. Milk proteins have buffering properties [11]. Hence, it is necessary to produce Feta-type cheese with whey-less method, which is a good substitute for ultrafiltrated cheeses [12]. Whey produced from cheese making, as a by-product, limits productivity. The production of whey can create additional costs for waste treatment, even though whey contains food-grade ingredients that have been separated from milk. In the production of cheese in the whey-less method, different proportions of milk protein concentrate, whey protein concentrate, sodium caseinate, whole milk powder, skimmed milk powder, fat replacers, cream, vegetable fat, and fresh milk are used, and the cheese is similar to ultrafiltrated cheese [12]. The objective of this study was to optimize the formulation of whey-less Feta-type cheese using milk protein concentrate, whey protein concentrate, and cream, and evaluate the effects of pomegranate juice on the characterization of whey-less Feta-type cheese.

2. Materials and Methods

2.1. Whey-Less Feta-Type Cheese Production

2.1.1. Raw Materials. Fresh milk was obtained from Holstein dairy cows on local farms in Shiraz. Cream and milk protein concentrate (MPC) were obtained from Fars Pegah Dairy Co. (Shiraz, Iran) and whey protein concentrate (WPC) was purchased From Glanbia Co. (Germany). Chymosin (Clerici Sacco International-Caglificio Clerici SpA, Italy) and culture (FEM Feferm, Optiferm GmbH, Germany) were used as rennet and starter, respectively.

2.1.2. Experimental Design. In the current investigation, the effects of MPC (5–15%), WPC (0–10%), and cream (45–55%) on the properties of whey-less cheese were evaluated by Simplex-Lattice mixture design. This experimental design was carried out in the form of 14 treatments. The levels of raw material for treatments are given in Table 1.

2.1.3. Cheese Processing. MPC and WPC powders and cream were added to fresh milk according to the experimental design. The mixtures were heated at 45°C for 1 h to hydrate the powders and homogenized at 50 bar through a single-stage valve homogenizer and pasteurized at 63°C for 30 min. After cooling to 35°C, they were transferred to cheese vats, and starter culture was added at a level of 10 units/1000 L and $CaCl_2$ at a level of $0.2\,g \cdot L^{-1}$ milk. The inoculated milk was held for 30 min at 35°C and salt (15 g/1000 L directly before the formation of the clot) and rennet (0.04 g/1000 L) were added to the milk. The mixtures were transferred to 100 g plastic containers and kept for 20 min at 35°C to form a coagulum. Finally, containers were heat sealed with aluminum coat. The cheese samples were incubated at 40°C for 4 h and then refrigerated at 6°C. After 48 hours, samples were subjected to different tests [13].

2.1.4. Optimization of Whey-Less Feta-Type Cheese Formulation. The samples of Whey-less Feta-type cheese were subjected to different tests. The optimization was carried out by desirability function and averages of the properties of market cheeses. Seven different brands of ultrafiltrated Feta-type cheese marketed in Iran were selected and their physicochemical and texture indices were measured in three replicates. Numerical optimization was performed with Design Expert®9.0 software and optimized percentages of raw materials were obtained.

2.2. Fruit Whey-Less Feta-Type Cheese Production

2.2.1. Pomegranate Juice Preparation. Pomegranate fruit cv. "Rabbab-e- Shiraz" was harvested from Qasro-Dasht Gardens of Shiraz. After washing the fruit and the manual granulation, the pomegranate juice was obtained by pressing the seeds and then filtering and pasteurizing (70°C for 10 min). Pomegranate juices were stored at −20°C.

2.2.2. Cheese Processing. To produce fruit Feta cheese, the optimal ratios of fresh milk, cream, MPC, and WPC powders were mixed together. According to the method of whey-less Feta-type cheese production, the mixture was homogenized, pasteurized, and cooled. Pomegranate juice was added to milk at 35°C. Then the culture, salt, $CaCl_2$, and rennet were added according to the whey-less Feta-type cheese production method. This mixture was transferred to 100 g

Table 1: Experimental design variables* and levels**.

Run	WPC (%)	MPC (%)	Cream (%)
1	3.33	8.33	48.3
2	6.67	6.67	46.7
3	0.00	10.0	50.0
4	0.00	5.00	55.0
5	5.00	5.00	50.0
6	5.00	10.0	45.0
7	0.00	15.0	45.0
8	10.0	5.00	45.0
9	1.67	11.67	46.7
10	0.00	15.0	45.0
11	10.0	5.00	45.0
12	0.00	10.0	50.0
13	0.00	5.00	55.0
14	1.67	6.67	51.7

*WPC: whey protein concentrate; MPC: milk protein concentrate. **g/100 g of cheese.

plastic containers and kept for 20 min at 35°C to form a coagulum and finally heat sealed with an aluminum coat. The cheeses were incubated at 40°C until their pH reached 4.8 and then placed in a refrigerator at 6°C for 60 days. Samples were evaluated using physicochemical, sensory, and microbial tests at 15 day intervals.

2.3. Physicochemical Analysis. Total solids, titratable acidity based on lactic acid (w/w), protein, ash, salt, and pH of samples were measured according AOAC [14]. Syneresis was measured by determining the weight ratio of whey to curd during the time of storage [15]. The surface color of the samples was determined by a digital Canon camera (Canon, Model IXUS 230 HS, 14.0 Megapixels, Tokyo, Japan). The resultant pictures were studied by Adobe Photoshop CS 6 Software and the main color parameters, such as L^*, a^* and b^*, were extracted. [16]. To evaluate the degree of proteolysis, nonprotein nitrogen index (NPN) was determined [17]. Lipolysis was evaluated by determining the acidity index. Fat was extracted from cheese samples using diethyl ether and their acidity values (mill equivalent per 100 grams of fat) were determined by titration with alcoholic potash [17]. To determine the thiobarbituric acid and the peroxide value, the fat was extracted using chloroform [18]. Thiobarbituric acid's value as a secondary oxidation product and peroxide value were determined based on the Kirk and Sawyer [19] method. To measure the total phenolic content and the total anthocyanin content and determine the antioxidant capacity of cheese samples, an aliquot of cheese (5 g) was mixed with methanol (40 ml) and placed on a shaker for 1 h at 65°C and then centrifuged for 15 minutes at 4°C. Supernatant was used as a cheese extract in antioxidant tests. The amount of total phenolic compounds present in the samples was investigated using the "Folin-Ciocalteu" method [20]. The total amount of anthocyanins was measured using the colorimetric method (absorbance difference at different pHs) [21]. To measure the antioxidant activity, DPPH solution at a concentration of 0.1 mM in methanol was used and the absorbance was read at 517 nm [20].

2.4. Texture Analysis. The textural properties were evaluated by a texture analyzer (CT3 Brookfield, USA). For this purpose, cheese samples were placed at room temperature for 10 min. Then, cylindrical specimens with a 27 mm diameter and 20 mm height were taken from cheese samples and placed in the texture analyzer and compressed by a probe with 35 mm diameter to 20% of their original height. The speed of the probe was 60 mm/min. Hardness, cohesiveness, adhesiveness, gumminess, chewiness, and springiness were determined from the curve [22].

2.5. Microbiological Analysis. Total counts were evaluated by plate count agar at 37°C for 48 h. The mesophilic and thermophilic lactobacilli were determined by MRS agar at 30°C and 45°C, respectively, for 48 h. Also, the mesophilic and thermophilic lactococci were enumerated by M17 agar at 30°C and 37°C, respectively, for 48 h [23].

2.6. Sensory Analysis. Sensory evaluation of samples was performed after 30 days. Samples were randomly coded and placed for 30 minutes at room temperature and then evaluated by a group of 20 trained panelists. Surface color, internal color, surface appearance, internal appearance, flavor, texture, and general acceptance were evaluated by the 5-point hedonic method (minimum and maximum satisfaction were rated 1 to 5, respectively) [12].

2.7. Statistical Analysis

2.7.1. Whey-Less Feta-Type Cheese Production. Design Expert®9.0 software was used to determine experimental design and analyze the data. Mixture design is defined as a spatial type of RSM in which the factors are the components of a mixture and the response varies as the proportions vary, i.e., the response is affected by the variation of the proportions. One of the most widely used mixture design types is the Simplex-Lattice design [24]. For each response, analysis of variance was conducted to determine significant differences among various treatments. The relationship between each of the responses with independent variables was modeled. In order to evaluate the validity of the fitted models, the values of adj-R^2 coefficients and lack of fit test were determined. Finally, optimization of whey-less Feta-type cheese, based on the physicochemical and textural properties of market cheeses, was done.

2.7.2. Fruit Whey-Less Feta-Type Cheese Production. The effects of pomegranate juice concentration on properties of whey-less Feta-type cheese were evaluated by a completely randomized design with at least 3 replications. SPSS V. 25 statistical software was used for ANOVA analysis of the results and Duncan post hoc test for multiple comparisons were done at a significant level of 0.05 ($p < 0.05$).

3. Results and Discussion

3.1. Whey-Less Feta-Type Cheeses' Properties. With the aid of data analysis regression analysis by using Design Expert®9.0 software, the properties of all cheeses were modeled in the form of a polynomial equation as a function of independent variables. Using ANOVA, the significance of the linear and interaction effects of regression model coefficients was investigated for each property. For evaluation of the validity of the fitted models, the values of adj-R^2 coefficients and lack of fit test were used. As shown in Table 2, adj-R^2 of the models for various responses ranged from 0.87 to 0.99, and they can be used to predict changes in these properties. The results showed that lack of fit for all properties measured at 95% confidence level was not significant. Therefore, the high adj-R^2 coefficient and not significant lack of fit for all properties confirm the accuracy of the model for fit information. The numerical value of the properties of cheese can be predicted by substituting different percentages of cream, WPC, and MPC in the equations.

3.2. Optimization of Whey-Less Feta-Type Cheeses (Numerical Optimization). One of the most popular and the most frequently used approaches to simultaneous optimization is the desirability function approach. Individual goals are combined into a single objective measure to be maximized using a geometric mean function. It is possible to obtain an overall desirability from the individual desirabilities [24]. The numerical value of desirability is between zero and one and its low level indicates that the target is not reachable, while its high level indicates that the target is fully met. When the goal is to optimize multiple responses simultaneously, the desirability of each of the responses is determined and their geometric mean is used as an indicator of the simultaneous fulfillment of the goals. Based on the physicochemical properties of market cheeses (Table 3), numerical optimization was performed. The optimal point with the highest desirability was obtained with percentage of independent variables of cream, MPC, and WPC at 45.6, 11.7, and 2.7%, respectively. The characteristics of the cheese at the optimum point were listed in Table 3.

3.3. Properties of Pomegranate Juice. The chemical properties of pasteurized pomegranate juice are shown in Table 4. The results of pomegranate juice analysis showed that total soluble solids, acidity, total ash, and pH were 18.12, 1.5, 0.29%, and 3.2, respectively. Akbarpour et al. [25] reported that total soluble solid, acidity, and pH of pomegranate juice obtained from Rabbab cultivar were 19.88%, 1.49%, and 3.09 respectively. Total phenol has been reported for Iranian pomegranate juices at 2960–9850 mg/L, for Turkish commercial pomegranate juices at 1080–9449 mg/L, and for Italian pomegranate juices at 1600–3730 mg/L [26]. The total phenol of pomegranate juice (2450 mg/L) in our study is within the range reported by previous researchers. The anthocyanin content of pomegranate juice was 270.82 mg/L in our study, while it was reported to be 81–369 mg/L in Turkish pomegranate juices, 2380–9300 mg/L in Iranian pomegranate juices, and 11–178 mg/L in Tunisia pomegranate juices [26].

3.4. Fruit Whey-less Feta-type Cheese Properties. Various characteristics of Feta-type cheese containing different pomegranate juice concentrations were analyzed during the storage time (60 days) at intervals of 15 days. The data were analyzed by SPSS software and the meanings were compared with Duncan's multiple range test. The results of the analysis showed that pomegranate juice has a very significant effect on all cheese properties ($p < 0.0001$). The pictures of Feta-type cheese containing different pomegranate juice concentrations on the first day after production are presented in Figure 1.

3.4.1. Physical and Chemical Properties. Physicochemical properties of cheese are shown in Table 5. The pH of cheese decreases as the amount of pomegranate juice increases. This is attributed to the presence of organic acids in pomegranate juice, such as citric acid and malic acid [27]. These results are consistent with the results of Salwa et al. [28]. With increasing percentage of pomegranate juice, the amount of whey increased. The syneresis process depends on the extensive redistribution of the casein network, after the formation of the initial gel. Redistribution is more intense at a higher temperature and lower pH [29]. During the time of storage, whey percentage was initially increased to the thirtieth day and then it showed a decreasing trend. Syneresis in the first weeks of storage is due to an increase in acidity and a decrease in pH, as well as increased casein cross-linking and excessive contraction of the crude due to cooling. After 30 days, the whey is again absorbed into the crude; this appears to be due to increased proteolysis, followed by an increase in lyophilic insides in matrix of cheese then water absorption increased [29].

The results showed that pomegranate juice reduced the dry matter of the cheese (Table 5). This decrease could be due to the high humidity of pomegranate juice; therefore, as the percentage of pomegranate juice in the formulation increased, more water is absorbed by the WPC and MPC in the cheese matrix, which decreases the dry matter. Initially, until the 30th day, the amount of dry matter increased and then showed a decreasing trend. The whey diagram also confirms the changes in dry matter.

Pomegranate juice significantly reduced the amount of salt. With the constant initial amount of salt, with increasing pomegranate juice and the subsequent moisture content of cheese, its percentage decreased from 1.37 in the control to 1.12 in cheese with 20% pomegranate juice. Pomegranate juice significantly decreased the ash and protein content of cheese. This decrease is due to low percentage of ash and protein content of pomegranate juice compared to the control sample.

Pomegranate juice reduces nonprotein nitrogen. NPN is the index of proteolysis process. This process is the most important factor in curing the different types of cheese. The proteases of the starter are the most important proteolytic enzymes found in whey-less cheese. Due to the fact that all

Table 2: Predictive models for characteristics of whey-less Feta cheese.

Responses	Predictive models	R^2
Syneresis (%)	$0.574A^{***} - 2.550B^{***} + 17.484C^{***} + 0.030\ AB^{ns} - 0.416\ AC^{***} - 0.148BC^{**}$	0.998
Dry matter (%)	$0.680A^{***} - 0.545B^{***} + 8.836C^{***} + 0.025AB^{ns} - 0.183AC^{***} - 0.054BC^{ns}$	0.939
Fat (%)	$0.474A^{***} - 0.046B^{***} + 4.950C^{***} - 0.0054AB^{ns} - 0.116\ AC^{***} - 0.026BC^{ns}$	0.991
Acidity (% lactic acid)	$0.025A^{***} - 0.142B^{***} + 0.022C^{***} - 0.0033\ AB^{*} - 0.000028AC^{ns} + 0.00026BC^{ns}$	0.946
Salt (%)	$0.018A^{***} + 0.096B^{***} - 0.124C^{***} - 0.001AB^{ns} + 0.0037AC^{ns} + 0.0017BC^{ns}$	0.939
Total protein (%)	$0.07A^{***} - 0.068B^{***} + 1.7C^{***} + 0.018\ AB^{ns} - 0.031AC^{*} - 0.018BC^{ns}$	0.984
pH	$0.075A^{***} + 0.424B^{***} + 0.455C^{***} - 0.0065\ AB^{ns} - 0.0067AC^{ns} - 0.0044BC^{ns}$	0.945
Ash (%)	$0.03A^{***} - 0.083B^{***} - 0.148C^{***} - 0.0046AB^{ns} + 0.0064\ AC^{ns} - 0.0075\ BC^{*}$	0.920
Hardness (g)	$13.4A^{***} + 676.8B^{***} + 181.4C^{***} - 14.0AB^{***} - 5.57AC^{ns} + 0.28BC^{ns}$	0.979
Cohesiveness	$0.0094\ A^{***} + 0.0036B^{***} - 0.025C^{***}$	0.867
Gumminess (g)	$6.47A^{***} + 304.9B^{***} - 80.5C^{***} - 6.27AB^{*} + 1.21\ AC^{ns} - 2.35BC^{ns}$	0.942
Chewiness (mj)	$0.125A^{***} + 1.24B^{***} + 0.446C^{***}$	0.869
Springiness (mm)	$0.026A^{***} + 0.270B^{***} - 1.28C^{***} - 0.00034AB^{ns} + 0.026AC^{ns} + 0.013BC^{ns}$	0.923
Adhesiveness (mj)	$0.084A^{***} + 0.41B^{***} - 0.055C^{***} - 0.014AB^{**} - 0.0031AC^{ns} + 0.014BC^{**}$	0.988
Color−L*	$1.035A^{***} + 2.89B^{***} + 0.718C^{***} - 0.039AB^{***} + 0.00075\ AC^{ns} + 0.040BC^{***}$	0.983
Color−a*	$0.072A^{***} + 0.18B^{***} - 0.214C^{***} - 0.0066AB^{*} + 0.0042AC^{ns} + 0.006BC^{ns}$	0.984
Color−b*	$0.39A^{***} + 0.51B\ ^{***} + 0.13C^{***} - 0.012AB^{ns} - 0.00033AC^{ns} + 0.017BC^{ns}$	0.979
Acceptance	$0.08A^{***} + 1.58B^{***} + 1.64C^{***} - 0.034AB^{*} - 0.040AC^{*} - 0.02BC^{ns}$	0.909

A: cream%, B: milk protein concentrate (MPC)%, C: whey protein concentrate (WPC)%. $^{***}p \le 0.001$, $^{**}p \le 0.01$, $^{*}p < 0.05$. ns, nonsignificant.

Table 3: Physicochemical characteristics of market Feta cheese and optimal cheese*.

Properties	Syneresis (%)	Dry matter (%)	Fat (%)	Acidity (%)	Salt (%)	Total protein (%)
Market cheese	0.21 ± 12.20^{a}	$37.10^{a} \pm 0.56$	0.35 ± 16.52^{a}	0.05 ± 1.18^{a}	0.03 ± 1.35^{a}	$12.20^{a} \pm 0.26$
Optimal cheese	$2.50^{b} \pm 0.71$	$37.40^{a} \pm 0.57$	$16.25^{a} \pm 0.37$	0.01 ± 1.09^{b}	$1.38^{a} \pm 0.03$	0.22 ± 12.35^{a}
Properties	Ash (%)	pH	Hardness (g)	Cohesiveness	Gumminess (g)	Chewiness (mj)
Market cheese	$3.45^{a} \pm 0.40$	$5.10^{a} \pm 0.12$	$1161^{a} \pm 50.00$	$0.430^{b} \pm 0.09$	$552.00^{b} \pm 33.6$	$16.25^{b} \pm 2.8$
Optimal cheese	$3.50^{a} \pm 0.70$	0.07 ± 4.75^{b}	$1109^{a} \pm 84.15$	$0.565^{a} \pm 0.17$	$625.75^{a} \pm 29.34$	$21.09^{a} \pm 1.64$
Properties	Springiness (mm)	Adhesiveness (mj)	Color-L	Color-b	Color-a	
Market cheese	$3.10^{a} \pm 0.43$	$2.3^{b} \pm 0.22$	$63.50^{a} \pm 0.42$	$17.00^{a} \pm 0.64$	$1.00^{b} \pm 0.02$	
Optimal cheese	$3.44^{a} \pm 0.50$	$1.05^{a} \pm 0.34$	$62.70^{a} \pm 0.424$	$17.90^{a} \pm 0.64$	$2.40^{a} \pm 0.15$	

*Averages of three replicates have been reported as mean ± standard deviation. Means within each column with different lowercase letters are significantly different ($p < 0.05$).

Table 4: Properties of pasteurized pomegranate juice*.

Ash (%)	Total soluble solids (%)	Acidity (%)	pH	Total phenol (mg/100 ml)	Anthocyanin (mg/100 ml)
0.29 ± 0.05	18.12 ± 0.1	0.02 ± 1.5	0.02 ± 3.2	12 ± 245	0.23 ± 27.82

*Averages of three replicates have been reported as mean ± standard deviation.

the added rennet remains in the whey-less cheese, rennet plays an important role in proteolysis and is responsible for the initial proteolysis of casein while the proteases of the initiating bacteria carry out further proteolysis and degrade the $\alpha\ s_1$. Hesari et al. [30] have confirmed the role of both factors in the proteolysis of Feta cheese. NPN decreases as the amount of pomegranate juice increases. Pomegranate juice also increases the moisture content of cheese, which in turn reduces the nonprotein nitrogen ratio.

3.4.2. Antioxidant Properties. Antioxidant properties of cheese are shown in Table 5. The pomegranate antioxidant activity is mostly due to the presence of ascorbic acid and phenolic compounds such as ponicagelin, ponicaline, gallic acid, ethanolic acid, and anthocyanins [31]. Polyphenolic compounds of pomegranate can eliminate and neutralize free radicals 3–15 times more than other antioxidants, such as vitamins C and E [8] The pomegranate juice analysis showed that it contains 245 ± 12 (mg/100 mL) of phenolic compounds. Zarban et al. [8] found that pomegranate juice contained 205 ± 20 (mg/100 mL) of phenolic compounds. With the increase in the percentage of pomegranate juice, the amount of phenolic compounds significantly increased; the results are similar to the findings of Trigueros et al. [32]. Phenolic compounds are able to react with proteins, which depend on their concentration, pH, and molecular weight. Low molecular weight phenolic compounds cannot produce strong cross-linking, but polymer types and high molecular weights are more active in cross-linking and are rapidly deposited by protein. Therefore, as the amount of pomegranate juice increased, the phenolic compounds were enhanced in cheese. Anthocyanins are extensively found in plant cell extracts and produce red, blue, and purple colors in

(a) (b) (c) (d) (e)

Figure 1: Pictures of surface Feta-type cheese containing different pomegranate juice concentrations. (a) 0%. (b) 5%. (c) 10%. (d) 15%. (e) 20%.

many fruits and vegetables. Anthocyanins interact with each other and other pigments and proteins through hydrophobic and hydrogen bonds [33]. Pomegranate juice contains 27.22 ± 0.23 (mg/100 mL) of anthocyanin. Therefore, it is expected that adding pomegranate juice to cheese causes the presence of this pigment in cheese.

The results showed that antioxidant activity significantly increased as the amount of pomegranate juice increased. Zarban et al. [8] found in their study that pomegranate juice has a higher inhibitory effect (96%) than other fruits. Han et al. [10] also found that using phenolic compounds in cheese could enhance its antioxidant properties; they found that cheese with 0.15 mg/mL phenolic compounds had significant antioxidant properties. Also, similar results were achieved in the case of yogurt containing pomegranate juice, and the antioxidant properties of yogurt were increased with an increase in the amount of pomegranate juice. Phenolic compounds react with casein and whey protein, and affect their functional characteristics; various reports confirm that proteins exhibit antioxidant properties by interacting with phenolic compounds.

3.4.3. Oxidative Stability. The amount of free fatty acids in cheese represents the development of lipolysis. Lipolysis plays an important role in the development of the flavor of cheese. During the preparation and maturing of cheese, milk fat was hydrolyzed by the natural lipase of milk, lipolytic enzymes of starter and nonstarter bacteria, and lipase of psychrotrophic bacteria. The natural lipases of the milk are sensitive to heat; therefore, they are inactive in cheeses made from pasteurized milk and the essential lipolysis agent in these cheeses will be the starter and other bacteria. According to the results (Table 5), control samples have the highest FFA Index, which indicates that the inhibitory effect of pomegranate juice on the lipolysis by starters. Peroxide is the primary product of fat oxidation, and malondialdehyde is one of the secondary products of fat oxidation that is produced from the hydroperoxides in the second stages of oxidation. Pomegranate juice significantly decreased the peroxide value and the TBA index of cheese.

3.4.4. Textural Properties. Textural properties of cheese are shown in Table 6. Pomegranate juice reduced the hardness, cohesiveness, gumminess, chewiness, and springiness, and increased the adhesiveness of the sample. Water molecules and fat globules are located within the three-dimensional protein networks; if the amount of water increases, then the network structure will become weakened and more susceptible to deforming during compression [34]. According to the results, it can be said that pomegranate juice reduces dry matter and consequently the water in the cheese is increased; therefore, hardness and the amount of energy required for chewing the cheese was reduced. Moisture increases the plasticity of the protein matrix and decreases its elasticity. In addition, a lower protein density network has more adhesiveness and less cohesiveness. The reason for the reduction of cohesiveness is the weakness of the internal bonds in the structure of higher moisture cheeses and the softer tissue. These cheeses are easily irreversible deformed in the presence of pressure [35].

3.4.5. Color Properties. The main pigment in pomegranate juice is anthocyanin. The stability of anthocyanins is largely influenced by the chemical structure and concentration; the presence of oxygen, light, sugars, and enzymes; and the presence of complex compounds, pH, ascorbic acid, and storage temperatures [36]. With an increase in the amount of pomegranate juice, the L and b significantly decreased and a significantly increased (Table 5). Therefore, it can be said that pomegranate juice reduces brightness and yellowness of the cheese and increases its redness due to the presence of anthocyanin compounds. The results were similar with the findings of Karaaslan et al. [37].

3.4.6. Microbial Properties. Microbial properties of cheese are shown in Table 7. The survival rate of mesophilic and thermophilic Lactobacillus and Lactococcus were affected by the pomegranate juice. The number of starter significantly decreased with an increase in the amount of pomegranate juice. According to the results, the count of mesophilic was higher. Trigueros et al. [32] and Ranadheera et al. [38] also found similar results in terms of the impact of pomegranate juice on the survival of the Lactobacillus starter. The compounds of pomegranate juice inhibit the growth of starters and are important factors in reducing their populations. The acidity of pomegranate juice, the presence of high concentrations of phenolic compounds in pomegranate juice, and the antimicrobial properties of the pomegranate extract, which are attributed to tannins and flavonoids, are also effective factors in reducing the survival of microbial population, especially Gram-positive bacteria.

Table 5: The physicochemical properties of Fruit whey less Feta-type cheeses produced using different percentages of pomegranate juice (S_0, S_5, S_{10}, S_{15}, and S_{20}, respectively, containing 0, 5, 10, 15, and 20% of pomegranate juice).

Variables	Days	S_0	S_5	S_{10}	S_{15}	S_{20}	*P* Treatment	*P* Ripening
pH	0	4.82^{Aa}	4.83^{Aa}	4.80^{Aa}	4.83^{Aa}	4.77^{Ba}	*	*
	15	4.78^{Ab}	4.75^{Bb}	4.68^{Cb}	4.78^{ABb}	4.68^{Cb}	*	
	30	4.68^{Ac}	4.60^{Bc}	4.65^{Cb}	4.67^{Dc}	4.58^{Ec}	*	
	45	4.66^{Ac}	4.58^{Bc}	4.55^{Cc}	4.58^{Bd}	4.44^{Dd}	*	
	60	4.57^{Ad}	4.50^{Bd}	4.52^{Bc}	4.53^{Be}	4.40^{Cd}	*	
Syneresis (%)	0	2.5^{Aa}	2.9^{Ba}	3.5^{Ca}	3.9^{Da}	4.2^{Ea}	*	*
	15	2.7^{Ab}	3.1^{Bab}	3.5^{Ca}	4.2^{Da}	4.6^{Eb}	*	
	30	3.0^{Ab}	3.4^{Bab}	3.7^{Ca}	4.1^{Da}	4.7^{Eb}	*	
	45	1.9^{Ac}	2.3^{Bc}	2.5^{Cb}	3.2^{Db}	3.4^{Ec}	*	
	60	0.0^{Ad}	0.0^{Ad}	0.3^{Bc}	0.73^{Cc}	1.7^{Dd}	*	
Dry matter (%)	0	37.45^{Aa}	37.02^{Ba}	36.45^{Ca}	35.69^{Da}	35.11^{Ea}	*	*
	15	37.50^{Aab}	37.08^{Ba}	36.49^{Ca}	35.72^{Dab}	35.24^{Eb}	*	
	30	37.81^{Ab}	37.22^{Bb}	36.55^{Cb}	35.78^{Db}	35.35^{Ec}	*	
	45	37.24^{Ac}	36.68^{Bc}	35.95^{Cc}	35.25^{Dc}	34.45^{Ed}	*	
	60	36.69^{Ad}	35.86^{Bd}	35.28^{Cd}	34.32^{Dd}	34.24^{Ee}	*	
Fat (%)	0	16.27^{Aa}	15.39^{Ba}	14.92^{Ca}	14.66^{Da}	14.29^{Ea}	*	*
	15	16.30^{Aa}	15.40^{Ba}	14.88^{Ca}	14.72^{Da}	14.35^{Ea}	*	
	30	16.38^{Aa}	15.47^{Ba}	15.00^{Ca}	14.81^{Da}	14.38^{Ea}	*	
	45	15.90^{Ab}	15.28^{Bb}	14.71^{Cb}	14.58^{Db}	14.19^{Eb}	*	
	60	15.60^{A}	15.04^{Bc}	14.46^{Cc}	14.21^{Dc}	13.64^{Ec}	*	
Salt (%)	0	1.37^{Aa}	1.35^{Ba}	1.32^{Ca}	1.16^{Da}	1.12^{Ea}	*	*
	15	1.36^{Ab}	1.33^{ABb}	1.30^{Ba}	1.13^{Cb}	1.08^{Db}	*	
	30	1.35^{Ab}	1.32^{Ab}	1.27^{Bb}	1.17^{Ca}	1.07^{Db}	*	
	45	1.39^{Ac}	1.36^{Ba}	1.31^{Ca}	1.21^{Dc}	1.14^{Ea}	*	
	60	1.45^{Ad}	1.40^{Bc}	1.34^{Cc}	1.27^{Dd}	1.20^{Ec}	*	
Ash (%)	0	3.55	3.40	3.26	3.18	3.04	*	ns
	15	3.48	3.54	3.24	3.12	3.15	*	
	30	3.45	3.39	3.32	3.09	3.07	*	
	45	3.49	3.36	3.24	3.07	3.06	*	
	60	3.41	3.30	3.16	3.10	2.99	*	
Total protein (%)	0	12.38^{Aa}	11.80^{Ba}	11.38^{Ca}	10.93^{Da}	10.32^{Ea}	*	*
	15	12.39^{Aa}	11.94^{Ba}	11.36^{Ca}	10.95^{Da}	10.31^{Ea}	*	
	30	12.43^{Aa}	11.95^{Ba}	11.39^{Ca}	11.00^{Da}	10.28^{Eb}	*	
	45	12.13^{Ab}	11.70^{Bb}	11.31^{Cb}	10.93^{Da}	10.10^{Ec}	*	
	60	12.06^{Ac}	11.50^{Bc}	10.88^{Cc}	10.58^{Db}	9.99^{Ec}	*	
Nonprotein nitrogen/total protein	0	2.26^{Aa}	2.05^{Ba}	1.90^{Ba}	1.85^{BCa}	1.80^{Ca}	*	*
	15	3.05^{Ab}	2.28^{Bb}	2.55^{Bb}	2.70^{Bb}	2.48^{Cb}	*	
	30	4.66^{Ac}	2.55^{Bc}	3.50^{Bc}	3.15^{Cc}	3.05^{Dc}	*	
	45	6.53^{Ad}	3.78^{Bd}	3.85^{Bd}	3.82^{Bd}	3.35^{Cd}	*	
	60	7.63^{Ae}	4.90^{Be}	4.35^{Ce}	4.03^{De}	3.85^{De}	*	
Total phenol (mg/g of cheese)	0	0.078^{Aa}	0.148^{Ba}	0.252^{Ca}	0.353^{Da}	0.443^{Ea}	*	*
	15	0.072^{Aa}	0.118^{Bb}	0.173^{Cb}	0.290^{Db}	0.378^{Eb}	*	
	30	0.036^{Ab}	0.067^{Bc}	0.093^{Cc}	0.225^{Dc}	0.293^{Ec}	*	
	45	0.024^{Ac}	0.027^{Ad}	0.063^{Bd}	0.147^{Cd}	0.203^{Dd}	*	
	60	0.014^{Ad}	0.016^{Ae}	0.049^{Be}	0.102^{Cc}	0.183^{De}	*	
Anthocyanin (mg/g of cheese)	0	0^{Aa}	0.013^{Ba}	0.023^{Ca}	0.038^{Da}	0.049^{Ea}	*	*
	15	0^{Aa}	0.007^{Bb}	0.018^{Cb}	0.034^{Db}	0.041^{Eb}	*	
	30	0^{Aa}	0.003^{Bc}	0.011^{Cc}	0.030^{Dc}	0.033^{Ec}	*	
	45	0^{Aa}	0^{Ad}	0.008^{Bd}	0.022^{Cd}	0.027^{Dd}	*	
	60	0^{Aa}	0^{Ad}	0.003^{Be}	0.018^{Ce}	0.024^{Dd}	*	
Radical inhibition %	0	5.54^{Aa}	20.20^{Ba}	30.33^{Ca}	42.10^{Da}	53.99^{Ea}	*	*
	15	7.03^{Ab}	18.00^{Bb}	27.16^{Cb}	34.92^{Db}	39.72^{Eb}	*	
	30	8.34^{Ac}	15.16^{Bc}	20.11^{Cc}	21.30^{Cc}	20.89^{Cc}	*	
	45	9.01^{Ad}	11.50^{Bd}	12.72^{Cd}	17.95^{Dd}	18.28^{Ed}	*	
	60	9.74^{Ae}	9.25^{Ae}	11.78^{Be}	15.36^{Ce}	17.33^{De}	*	

Table 5: Continued.

Variables	Days	S_0	S_5	S_{10}	S_{15}	S_{20}	*P* Treatment	*P* Ripening
Ferry fatty acid (meq/100 g fat)	0	0.30^{Aa}	0.27^{Ba}	0.25^{Ca}	0.20^{Da}	0.19^{Da}	*	
	15	0.35^{Ab}	0.28^{Ba}	0.27^{Bab}	0.22^{Ca}	0.20^{Cb}	*	
	30	0.51^{Ac}	0.29^{Ba}	0.28^{Bb}	0.25^{Cb}	0.24^{Cc}	*	*
	45	0.65^{Ad}	0.32^{Bb}	0.30^{BCc}	0.28^{Cc}	0.27^{Cd}	*	
	60	0.78^{Ae}	0.36^{Bc}	0.33^{Cd}	0.32^{Cd}	0.33^{Ce}	*	
Peroxide value (meq g o_2/kg fat)	0	0.130^{Aa}	0.125^{ABa}	0.120^{BCa}	0.115^{Da}	0.095^{Ea}	*	
	15	0.150^{Ab}	0.150^{Ab}	0.150^{Ab}	0.130^{Bb}	0.135^{Bb}	*	
	30	0.200^{Ac}	0.195^{Ac}	0.190^{Ac}	0.170^{Bc}	0.150^{Cc}	*	*
	45	0.250^{Ad}	0.245^{Ad}	0.240^{Ad}	0.220^{Bd}	0.225^{Bd}	*	
	60	0.310^{Ae}	0.300^{Ae}	0.295^{Ae}	0.275^{Be}	0.250^{Ce}	*	
Thiobarbituric acid index (ppm)	0	0.195^{Aa}	0.180^{ABa}	0.175^{Ba}	0.150^{Ca}	0.070^{Da}	*	
	15	0.575^{Ab}	0.450^{ABb}	0.500^{Bb}	0.410^{Cb}	0.220^{Db}	*	
	30	1.015^{Ac}	1.115^{Ac}	0.940^{Bc}	0.925^{Cc}	0.800^{Dc}	*	*
	45	1.650^{Ad}	1.700^{ABd}	1.550^{Bd}	1.425^{Cd}	1.250^{Dd}	*	
	60	2.135^{Ae}	1.990^{Be}	1.865^{Ce}	1.815^{De}	1.470^{Ee}	*	
L*	0	62.25^{Aa}	57.55^{Ba}	55.30^{Ca}	53.95^{Da}	51.30^{Ea}	*	
	15	61.75^{Ab}	56.55^{Bb}	54.60^{Cb}	52.90^{Db}	50.50^{Eb}	*	
	30	60.50^{Ac}	55.60^{Bc}	54.10^{Cb}	52.45^{Db}	50.80^{Eb}	*	*
	45	59.05^{Ac}	55.10^{Bc}	53.80^{Cc}	50.95^{Dc}	49.00^{Dc}	*	
	60	58.35^{Ad}	54.25^{Bd}	52.75^{Bd}	50.10^{Cc}	48.35^{Ed}	*	
b*	0	17.45^{Ad}	15.65^{Bd}	14.95^{Bd}	14.05^{Cc}	13.20^{Dd}	*	
	15	18.05^{Ac}	15.90^{Bc}	15.10^{Bc}	14.10^{Cbc}	13.45^{Dc}	*	
	30	18.20^{Ab}	16.15^{Bbc}	15.50^{Bc}	14.55^{Cb}	13.70^{Dc}	*	*
	45	18.50^{Ab}	16.45^{Bb}	16.60^{Bb}	15.20^{Ca}	14.15^{Db}	*	
	60	19.65^{Aa}	16.95^{Ba}	17.20^{Ba}	15.15^{Ca}	14.40^{Da}	*	
a*	0	2.50^{Ea}	4.10^{Da}	5.75^{Ca}	7.30^{Ba}	7.75^{Aa}	*	
	15	2.35^{Eb}	3.55^{Db}	5.15^{Cb}	7.20^{Bb}	7.40^{Ab}	*	
	30	2.35^{Eb}	3.25^{Db}	4.55^{Cc}	6.70^{Bc}	7.20^{Ac}	*	*
	45	2.05^{Ec}	2.45^{Dc}	4.50^{Cc}	5.65^{Bd}	7.20^{Ac}	*	
	60	1.80^{Ed}	2.30^{Dc}	3.65^{Cd}	5.40^{Bd}	5.85^{Ad}	*	

ns, nonsignificant, * $p \leq 0.05$. Means within each row with different uppercase letters are significantly different ($p \leq 0.05$), and means within each column with different lowercase letters are significantly different ($p < 0.05$).

Table 6: The textural properties of Fruit whey less Feta-type cheeses produced using different percentages of pomegranate juice (S_0, S_5, S_{10}, S_{15}, and S_{20}, respectively, containing 0, 5, 10, 15, and 20% of pomegranate juice).

Variables	Days	S_0	S_5	S_{10}	S_{15}	S_{20}	*P* Treatment	*P* Ripening
Hardness (g)	0	1038^{Aa}	826.0^{Ba}	599.5^{Ca}	587.5^{Ca}	476.5^{Da}	*	
	15	1079^{Ab}	860.0^{Bb}	760.0^{Cb}	732.5^{Cb}	510.0^{Db}	*	
	30	1034^{Ab}	952.5^{Bc}	778.5^{Cb}	695.0^{Cb}	539.5^{Db}	*	*
	45	983.5^{Aa}	801.0^{Ba}	644.5^{Cc}	641.5^{Cc}	384.0^{Dc}	*	
	60	944.5^{Ac}	727.0^{Bd}	619.0^{Ca}	540.0^{Da}	302.8^{Ed}	*	
Cohesiveness	0	0.555^{Aa}	0.530^{Ba}	0.380^{Ca}	0.390^{Ca}	0.350^{Ca}	*	
	15	0.618^{Ab}	0.525^{Bb}	0.435^{Cb}	0.415^{Cb}	0.475^{Cb}	*	
	30	0.625^{Ab}	0.555^{Bb}	0.550^{Bb}	0.490^{Cb}	0.425^{Cb}	*	*
	45	0.550^{Aac}	0.415^{Bc}	0.365^{Cac}	0.380^{Cac}	0.340^{Cac}	*	
	60	0.410^{Ad}	0.415^{Ac}	0.305^{Cd}	0.305^{Cd}	0.280^{Cd}	*	
Gumminess (g)	0	591.5^{Aa}	436.6^{Bab}	282.5^{Cab}	237.5^{Dab}	188.3^{Eab}	*	
	15	615.4^{Aa}	437.5^{Bb}	325.0^{Cb}	253.5^{Db}	190.7^{Eb}	*	
	30	592.5^{Aa}	441.0^{Bb}	295.5^{Cb}	252.5^{Db}	205.0^{Eb}	*	*
	45	525.0^{Ab}	432.5^{Bb}	322.5^{Cb}	272.5^{Db}	204.0^{Eb}	*	
	60	517.0^{Ac}	395.0^{Bb}	282.0^{Ca}	227.5^{Da}	160.5^{Ea}	*	
Chewiness (mj)	0	44.29^{Aa}	37.56^{Aa}	33.00^{Ba}	25.00^{Ba}	17.16^{Ca}	*	
	15	34.50^{Aab}	33.53^{Aab}	29.14^{Ba}	23.50^{Bab}	17.15^{Cab}	*	
	30	29.06^{Aab}	32.00^{Aab}	26.00^{Bb}	25.50^{Bab}	15.11^{Cab}	*	*
	45	23.64^{Ab}	31.25^{Ab}	25.00^{Bb}	21.50^{Bb}	12.25^{Cb}	*	
	60	25.00^{Ab}	30.5^{Ab}	21.00^{Bd}	15.115^{Bc}	11.00^{Cb}	*	

Table 6: Continued.

Variables	Days	S_0	S_5	S_{10}	S_{15}	S_{20}	P Treatment	P Ripening
Springiness (mm)	0	4.0	3.7	3.3	3.2	3.1	*	ns
	15	3.8	3.5	3.3	3.0	3.0	*	
	30	3.5	3.5	3.1	3.3	2.8	*	
	45	3.5	3.5	3.2	3.2	2.7	*	
	60	3.3	3.4	3.2	3.1	2.6	*	
Adhesiveness (mj)	0	1.00^{Aa}	1.37^{Ba}	1.56^{Ba}	1.69^{Ca}	1.95^{Ca}	*	*
	15	1.00^{Aa}	1.35^{Ba}	1.52^{Ba}	1.70^{Ca}	1.89^{Ca}	*	
	30	1.25^{Ab}	1.52^{Bb}	1.53^{Bab}	1.93^{Cb}	2.06^{Cab}	*	
	45	1.23^{Ab}	1.55^{Bb}	1.35^{Bab}	2.15^{Cb}	2.15^{Cab}	*	
	60	1.20^{Ab}	1.65^{Bb}	1.75^{Bb}	2.07^{Cb}	2.38^{Db}	*	

ns, nonsignificant, $^*p \le 0.05$. Means within each row with different uppercase letters are significantly different ($p < 0.05$), and means within each column with different lowercase letters are significantly different ($p < 0.05$).

Table 7: The microbial properties of Fruit whey less Feta-type cheeses produced using different percentages of pomegranate juice (S_0, S_5, S_{10}, S_{15}, and S_{20}, respectively, containing 0, 5, 10, 15, and 20% of pomegranate juice).

Variables	Days	S_0	S_5	S_{10}	S_{15}	S_{20}	P Treatment	P Ripening
Mesophilic lactobacilli ($\log_{10}$ cfu/g)	0	8.43^{Aa}	8.33^{Ba}	7.65^{Ca}	7.15^{Da}	6.59^{Ea}	*	*
	15	8.28^{Aa}	8.23^{Aa}	7.58^{Ba}	7.03^{Ca}	6.49^{Db}	*	
	30	8.06^{Ab}	7.58^{Bb}	7.38^{Cb}	6.88^{Db}	6.48^{Eb}	*	
	45	7.82^{Ac}	7.50^{Bb}	6.89^{Cc}	6.43^{Dc}	6.30^{Ec}	*	
	60	7.47^{Ad}	7.11^{Bc}	6.24^{Cd}	6.12^{Dd}	5.84^{Ed}	*	
Thermophilic lactobacilli ($\log_{10}$ cfu/g)	0	7.54^{Aa}	7.03^{Ba}	6.95^{Ba}	6.09^{Ca}	5.99^{Da}	*	*
	15	7.14^{Ab}	6.93^{Bb}	6.88^{Bb}	5.78^{Cb}	5.54^{Db}	*	
	30	6.89^{Ac}	6.10^{Bc}	6.11^{Bc}	5.87^{Cb}	5.47^{Dc}	*	
	45	6.75^{Ad}	5.70^{Ad}	5.86^{Ad}	5.50^{Bc}	5.23^{Cd}	*	
	60	6.12^{Ae}	5.24^{Be}	5.08^{Ce}	5.06^{Cd}	4.91^{De}	*	
Mesophilic lactococci ($\log_{10}$ cfu/g)	0	8.42^{Aa}	8.11^{Aa}	7.62^{Ba}	7.27^{Ca}	6.64^{Da}	*	*
	15	8.41^{Aa}	8.24^{Aa}	7.57^{Ba}	7.11^{Ca}	6.40^{Da}	*	
	30	7.73^{Ab}	7.79^{Ab}	7.10^{Bb}	6.732^{Cb}	6.00^{Db}	*	
	45	7.46^{Ac}	7.34^{Ac}	6.94^{Bc}	6.42^{Cc}	5.49^{Dc}	*	
	60	7.05^{Ad}	6.83^{Ad}	6.50^{Bd}	6.02^{Cd}	5.06^{Dd}	*	
Thermophilic lactococci ($\log_{10}$ cfu/g)	0	8.40^{Aa}	7.70^{Ba}	7.00^{Ca}	6.84^{Ca}	6.60^{Ca}	*	*
	15	8.18^{Ab}	7.13^{Bb}	6.53^{Cb}	6.53^{Cb}	6.46^{Cb}	*	
	30	7.45^{Ac}	7.07^{Bc}	6.11^{Cc}	6.01^{Cc}	6.02^{Cc}	*	
	45	7.15^{Ad}	6.40^{Bd}	5.09^{Cd}	5.22^{Cd}	5.29^{Cd}	*	
	60	6.935^{Ae}	5.93^{Be}	4.88^{Ce}	4.54^{Ce}	4.43^{Ce}	*	

ns, nonsignificant, $^* p \le 0.05$. Means within each row with different uppercase letters are significantly different ($p < 0.05$), and means within each column with different lowercase letters are significantly different ($p < 0.05$).

3.4.7. Sensory Attributes. One of the important characteristics of a food product is consumer acceptability. In this test, the surface color, internal color, surface appearance, internal appearance, texture, flavor, and acceptance of the samples were evaluated (Figure 2). The sample containing 20% of pomegranate juice has the highest rating of acceptance, surface color, internal color, and surface appearance and taste. In all cases, the sensory scores for the color of the internal texture was less than the surface color of the cheese. The surface appearance of samples was examined for porosity and flatness. The internal surface of the sample was examined after cutting, in order to investigate its porosity, smoothness, and homogeneity. Results indicated that the internal appearance of the samples were similar. The texture score of the samples showed that the more the pomegranate juice, the lower the score of the tissue. The findings of the texture analysis (Table 6) also confirmed the loss of texture in the sample with 20% pomegranate juice. The flavor of cheese is influenced by the formulation components and the complex biochemical reactions during storage that are carried out by the enzymes present in milk, cheese, and natural microorganisms and starters. Proteolysis, lipolysis, and glycolysis are major reactions that occur during storage. The proteolysis process has the greatest effect on flavor due to the production of free peptides and amino acids that contain flavoring compounds, such as amines, acids, thiols, and thioesters [39]. According to the panelists, samples containing 15 and 20% pomegranate juice have the highest flavor score, which suggests that the flavor of pomegranate juice cheese was pleasant and more acceptable for the panelists.

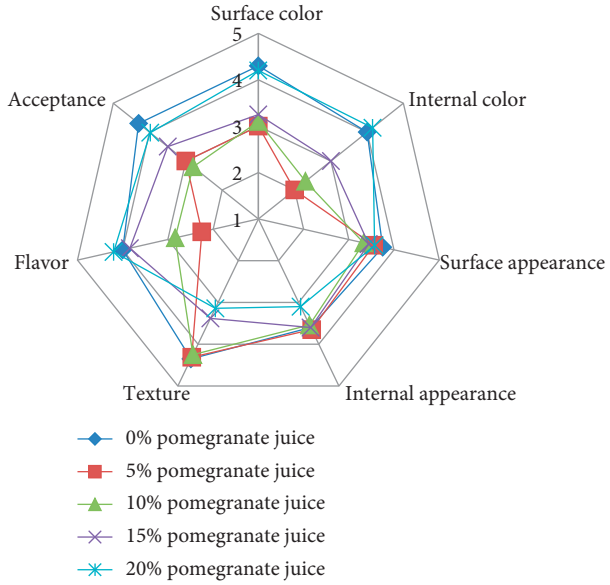

Figure 2: Sensory properties of Fruit whey-less Feta-type cheeses.

4. Conclusion

In order to produce whey-less cheese, MPC, WPC, and cream were mixed with fresh milk in different proportions, and optimization of its formula was developed by using Simplex-Lattice mixture design. The percentages of cream, MPC, and WPC at the optimal point were 45.6, 11.7, and 2.7%, respectively. Addition of Pomegranate juice to the optimal whey-less Feta cheese changed its physicochemical and textural properties. This cheese has better antioxidant properties and oxidative stability, but its texture was weaker than the control. Further studies are necessary to improve the texture of the Fruit Feta-type cheese containing pomegranate juice (20%). In general, it is recommended that cheese manufacturers produce fruit cheeses such as pomegranate cheese for people who are not interested in ordinary cheeses.

Data Availability

The data used to support the findings of this study are included within the article, and the raw data are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Inhibitory Effects of *Litsea cubeba* Oil and Its Active Components on *Aspergillus flavus*

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Aspergillus flavus (*A. flavus*) is a frequent harmful fungal pathogen. It can infect traditional Chinese medicine materials and release aflatoxin, to cause both economic and human health effects. By comparing the inhibitory potential of *Litsea cubeba* oil and its active components to *A. flavus* CGMCC 3.4408, citral was confirmed to be the main component that inhibits the growth of *A. flavus* CGMCC 3.4408, and the EC50 was 163.65 mg L^{-1}. The inhibitory effect of citral on *A. flavus* CGMCC 3.4408 was studied for colony growth rate, mycelium biomass, aflatoxin production, and microstructure. Citral slowed down the growth rate of colonies and reduced mycelium biomass and toxin production. Moreover, citral altered the morphology of fungal spores and mycelium. In addition, citral also has the inhibitory effects on the isolates of *A. flavus* from moldy traditional Chinese medicinal materials. Thus, citral can be used as a potential agent to check the growth of *A. flavus* or related fungal strains.

1. Introduction

Aspergillus flavus (*A. flavus* or mildew) is a pathogenic fungus that exists widely in nature and its metabolite aflatoxin B1 (AFB1) is highly carcinogenic [1]. *A. flavus* easily infects huge number of important agricultural products, medicinal materials, and much more to count [2]. In China, traditional Chinese medicine prevails to large extent; hence the production and use of medicinal materials are quite frequent [3]. Some Chinese medicinal materials are easy to breed mildew due to poor storage environment and their own matrix conditions [4]. *A. flavus* is the dominant fungus that causes mildew of medicinal materials [5]. Moldy medicinal materials not only cause great economic losses, but also have plenty of food safety risks. Therefore, it is a dire need to identify certain novel ways to inhibit the growth of *A. flavus* in particular, which infects Chinese medicinal materials.

Multiple strategies have been designed to control fungal growth and aflatoxin production [6, 7]. However, there is a global trend on limiting chemical fungicides application in grains and food products due to their toxicity and persistence, as the fungicide residues migrate into the food chain, leading to serious health hazards for consumers [8, 9]. Recently, many plant-based natural products which are safer compared to synthetic products have been considered as an alternative to these synthetic fungicides and preservatives [10–12]. Preventing *A. flavus* infections could be done by using plant essential oils, as they are classified as Generally Recognized As Safe (GRAS) and have low risk for developing resistance to pathogenic microorganisms [13]. *Cymbopogon citratus* essential oil, *Zingiber officinale* essential oil, and *Satureja hortensis* essential oil [14] are reported for significant reduction of *A. flavus* growth.

Litsea cubeba (Lour.) Pers. is an endemic Chinese plant and is rich in phytochemicals [15]. It is mainly grown in different provinces of China (Hunan, Sichuan, and Jiangxi). A large amount of essential oils can be extracted from the fruits and leaves of *Litsea cubeba* [16]. Different studies showed that *Litsea cubeba* oil can effectively inhibit plant pathogenic fungi and bacteria [17, 18]. It is a green and natural plant source as bacteriostatic agent. About 60–70% of *Litsea cubeba* oil comprised of citral, and researchers have focused on the bacteriostatic properties of citral [19, 20]. However, other components such as citronellal, α-terpineol, and linalool also have certain bacteriostatic properties but have not been studied well [21].

In the current study, sensitivity experiments and toxicity equations were designed to compare the inhibitory effects of *Litsea cubeba* oil, citral, citronellal, α-terpineol, and linalool on *A. flavus* CGMCC 3.4408 qualitatively and quantitatively. Then, the effects of citral on the colony growth, the biomass, toxicity, and microstructure of *A. flavus* CGMCC 3.4408 shall be investigated systematically. In order to evaluate the potential of citral as a mildew preventive agent for Chinese medicinal materials, the strains of *A. flavus* shall be isolated and identified from moldy traditional Chinese medicinal materials. The virulence of different strains and inhibitory ability of citral shall be analyzed to lay a foundation for the follow-up studies.

2. Materials and Methods

2.1. Materials. *A. flavus* CGMCC 3.4408 was bought from China Center of Industrial Culture Collection. The strain was scratched on potato dextrose agar (PDA) medium and cultured at 26°C for a period of time.

Litsea cubeba oil and citral were bought from factory (Jiangxi Mashan Chemical Co., Ltd.). Citronellal, α-terpineol, linalool, and other organic reagents were bought from Aladdin.

Moldy traditional Chinese medicinal materials used are Aurantii fructus and *Alisma orientale*, which were achieved from medicinal material base.

2.2. Preparation of A. flavus Spore Suspension. The culture dish of *A. flavus* that grew yellow-green spores was selected. About 5 mL sterile water (containing tween-80 with the volume ratio of 1%) was taken in the dish. Then, the spores were scraped off gently with the inoculation ring. The sterile water containing spores was transferred to the centrifuge tube containing glass beads. The solution was shaken for 1 min. Then, the impurities and hyphae were filtered out, and the filtrate was collected for use. The spores were counted using a blood cell counting plate and a microscope.

2.3. Inhibitory Effect of Litsea cubeba Oil and Active Components on A. flavus 3.4408

2.3.1. Determination of the Sensitivity. The bacteriostatic circle method was used in this study. The spore suspension was added to the sterilized medium, making the final concentration of spore suspension of 10^5–10^6 CFU·mL^{-1}. Then, about 25 mL was poured to each medium dish. After medium solidification, a central hole (6 mm) was made. 30 μL compounds whose concentrations were 150 mg mL^{-1}, 225 mg mL^{-1}, and 300 mg mL^{-1} were added to the hole, respectively, and cultivated under 26°C for 40 h. Finally, bacteriostatic circle diameter was measured.

2.3.2. Inhibitory Effect of Litsea cubeba Oil and Active Components on Spores. Spore counting method was used in this study. Compounds of different concentrations were prepared. Compound (1 mL) was mixed with 24 mL medium and then poured into a flat plate, so that the final concentration of the compound would be 90 mg L^{-1}, 180 mg L^{-1}, 360 mg L^{-1}, 720 mg L^{-1}, and 1440 mg L^{-1}. 100 μL, 10^6 CFU·mL^{-1} spore suspension was uniformly coated on each dish. Then, the numbers of colonies were calculated after culture for 40 h at 26°C. The experiment was repeated for three times.

2.4. Effects of Citral on the Growth of A. flavus 3.4408 in Solid Medium

2.4.1. Effects of Citral on the Growth Rate of Colony. Point contact method was used in this experiment. 1×10^5 CFU spores were inoculated in the culture medium center and cultured at 26°C for 24 h. Then, filter papers containing citral whose contents were 5 μL and 10 μL were pasted on the inner cover of the dish, respectively. The dishes were cultured at 26°C for 5 days. The colony diameter of *A. flavus* was measured every day. The linear equation between the colony diameter and time was analyzed. The slope was as the growth rate. The colony growth rate was calculated, and the influence of citral on the colony growth and morphology was analyzed.

2.4.2. Effects of Citral on A. flavus Microstructure. After 5 days of incubation in Section 2.4.1, 25 mm^2 cubes were cut from the culture medium and then completely soaked in 5% glutaraldehyde solution. Then, through a series of pretreatments, the colony of *A. flavus* spores and hyphae were observed by using transmission electron microscope (SEM).

2.5. Effects of Citral on Growth and Toxicity of A. flavus 3.4408 in Liquid Medium. Five conical flasks containing 25 mL medium were added to spore suspension (the final concentration of spore suspension was 10^5 CFU·mL^{-1}). Then, different concentration of citral was added to conical flask. The final concentrations of citral were 0 mg L^{-1}, 45 mg L^{-1}, 180 mg L^{-1}, 360 mg L^{-1}, and 720 mg L^{-1}, respectively. *A. flavus* was cultured at 26°C for 6 days. After culture, the hyphae were collected by suction filtration and weighed after desiccation. The filtrate was measured using aflatoxin B_1 detection kit (MeiZheng Biotechnology Co., Ltd.).

2.6. Effects of Citral on A. flavus from Moldy Chinese Medicinal Materials

2.6.1. Isolation and Identification of A. flavus from Moldy Chinese Medicinal Materials. The moldy Chinese medicinal materials were collected and eluted with sterile water. The eluted water points were placed on medium by spotting. Then, the dishes were cultured at 26°C for 1-2 days. The single colonies were transferred to new dishes. After culture of single colonies for a period of time, mycelia were collected with sterilized dissecting blade and then crushed by grinding and added into solvent to extract DNA. The DNA of the suspected strain was sequenced by Tsingke Biological Technology Co., Ltd.

2.6.2. Determination of Toxicity of A. flavus from Chinese Medicinal Materials. Conical flask containing 25 mL medium was taken and added with spore suspension whose final concentration of spore suspension was 10^5 CFU·mL^{-1}. The samples were incubated at 26°C for 6 days. After the culture period, the hyphae were collected by suction filtration and were weighed after desiccation. The content of AFB1 was detected using liquid chromatography.

2.6.3. Inhibitory Effect of Citral on A. flavus from Chinese Medicinal Materials. The bacteriostatic circle method and spore counting method were used (refer to the method of Section 2.3) to evaluate the inhibitory effect of citral on *A. flavus* that was isolated from moldy traditional Chinese medicine materials.

2.7. Statistical Analysis

2.7.1. Establishment of Toxicity Regression Equation. The experimental data in Section 2.3.2 was analyzed using DPS 7.05. In professional edition, the toxicity regression equation was established. Detailed method was that the concentration of the compounds was converted into logarithm and the inhibition rate was converted into probability. So the monadic linear regression relationship was developed between concentration and inhibition rate. Through the analysis of the significant linear relationship, the compounds can be compared to the strength of the pathogen virulence, and half effectively restrain mass concentration (EC 50) which was the important indicator of drug toxicity could be calculated.

2.7.2. Sequence Analysis and Establishment of Phylogenetic Trees. The resulting sequence in Section 2.6.1 was verified with Bioedit V7.0.9. The sequence was compared with NCBI, and the reliable sequence was selected and downloaded to establish the matrix. MEGA5.0 software was used for sequence alignment and pruning, and the first part was cut and saved for the construction of development trees. The phylogenetic tree was established using the maximum likelihood method using RaxML V7.2.6 software.

2.7.3. Significance Analysis. Significance analysis of the data was performed using one-way ANOVA (SPSS Statistics 21.0, SPSS Inc. Chicago, USA). Differences were considered significant when $P < 0.05$.

3. Results

3.1. Screening of Highly Effective Inhibitory Components in Litsea cubeba Oil. The sensitivity of *Litsea cubeba* oil to *A. flavus* 3.4408 was compared with that of citral, citronellal, α-terpineol, and linalool (Table 1). In general, the sensitivity of *Litsea cubeba* oil, citral, and citronellal was higher than that of α-terpineol and linalool. When the concentration of the compounds was 150 mg L^{-1}, *Litsea cubeba* oil and citronellal showed high sensitivity to *A. flavus*, while citral showed moderate sensitivity. At high concentration, the sensitivity of *Litsea cubeba* oil, citral, and citronellal was the same. The qualitative test showed that *Litsea cubeba* oil and its active components had inhibitory effect on *A. flavus*, but the inhibitory effect was different.

According to the results of spore counting method (Figure 1), *Litsea cubeba* oil and citral could completely inhibit the growth of *A. flavus* 3.4408 at high concentration. When the concentration of citral was 720 mg L^{-1}, the inhibition rate of *A. flavus* reached 100%, and the inhibitory effect was obviously better than that of α-terpineol and linalool (inhibitory rate was less than 50%). The establishment of toxicity equation is an effective method to quantitatively measure the inhibitory strength of active substances. The EC 50 is an important index to measure the antibacterial ability of compounds. Although there was no significant difference in the sensitivity of *Litsea cubeba* oil, citral, and citronellal to *A. flavus*, the EC 50 of *Litsea cubeba* oil and citral were significantly lower than that of citronellal (759.37 mg L^{-1}), which was 233.10 mg L^{-1} and 163.65 mg L^{-1}, respectively (Table 2).

The results of sensitivity test and spore counting test showed that citral had the best inhibitory effect on *A. flavus* 3.4408, and it was reported that the proportion of citral in *Litsea cubeba* oil was 60–70%, which was the main antibacterial component in *Litsea cubeba* oil.

3.2. Effects of Citral on the Growth of A. flavus 3.4408 in Solid Medium. The change trend of the colony diameter of *A. flavus* 3.4408 along with the culture days in the whole culture process under the treatment of citral is shown in Figure 2. The bacterial colonies showed differences on the first day of culture. With the increase of culture time, the overall growth trend of bacterial colony diameter was slower, and the inhibition effect of citral on the growth of *A. flavus* was gradually enhanced. At the same culture time, the higher the citral concentration was, the smaller the colony diameter of *A. flavus* was. At the 5th day of culture, the colony diameters of *A. flavus* were 82.27% and 72.72% compared to the control group under the citral concentrations of 5 μL and 10 μL, respectively. A linear equation (on the right side of Figure 2) with the slope of growth rate was obtained by plotting colony diameter against time. The growth rate of

Table 1: The sensitivity of *Litsea cubeba* oil and its active components.

Components	Concentration ($mg \cdot L^{-1}$)		
	150	225	300
Litsea cubeba oil	$16.03 \pm 0.71\ mm^{c**}$	$20.10 \pm 0.78\ mm^{a****}$	$26.93 \pm 0.55\ mm^{a****}$
Citral	$14.83 \pm 0.25\ mm^{c*}$	$26.93 \pm 0.45\ mm^{b****}$	$30.67 \pm 0.42\ mm^{b****}$
Citronellal	$16.17 \pm 1.26\ mm^{a**}$	$19.13 \pm 0.42\ mm^{c****}$	$28.83 \pm 1.04\ mm^{c****}$
α-Terpineol	$12.47 \pm 0.50\ mm^{b*}$	$17.00 \pm 0.50\ mm^{d*}$	$18.63 \pm 0.40\ mm^{d*}$
Linalool	$9.23 \pm 0.25\ mm^{c*}$	$15.3 \pm 0.36\ mm^{e**}$	$17.07 \pm 0.31\ mm^{e**}$

Inhibitory zone diameter > 20 mm, extreme sensitivity****; inhibitory zone diameter 15–20 mm, high sensitivity**; inhibitory zone diameter 10–15 mm, moderate sensitivity*; inhibitory zone diameter < 10 mm, low sensitivity*. Capital letters in each column showed significant differences ($P < 0.05$).

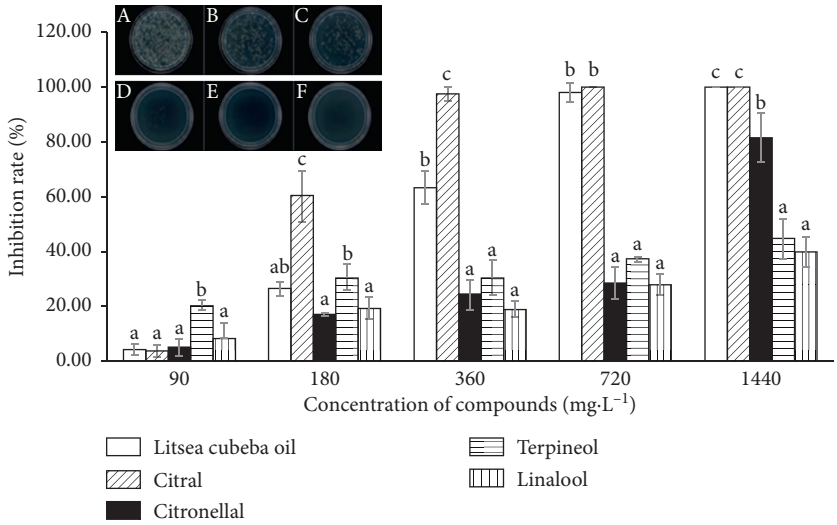

Figure 1: Spore counting method of *Litsea cubeba* oil and active components. ∗A: control (untreated); B: citral at 90 mg L^{-1}; C: citral at 180 mg L^{-1}; D: citral at 360 mg L^{-1}; E: citral at 720 $mg \cdot L^{-1}$; F: citral at 1440 mg L^{-1}. ∗a, b, and c show significant differences ($P < 0.05$).

Table 2: The toxicity equation of *Litsea cubeba* oil and active components.

Components	Toxicity equation	Correlation coefficient R^2	EC 50 ($mg \cdot L^{-1}$)
Litsea cubeba oil	$y = 5.3493x - 7.6646$	0.9704	233.10
Citral	$y = 6.0740x - 8.4473$	0.9741	163.65
Citronellal	$y = 1.7972x - 0.1768$	0.9278	759.37
α-Terpineol	$y = 0.5205x + 3.2106$	0.9655	2741.43
Linalool	$y = 0.8362x + 2.0715$	0.9636	3178.62

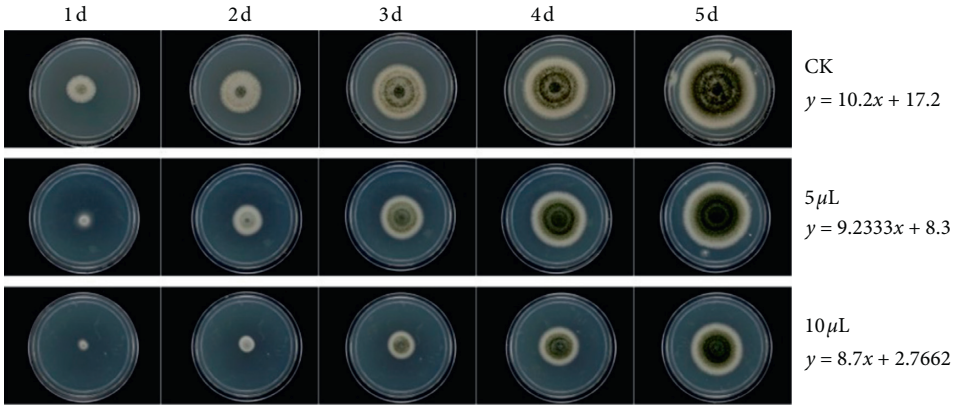

Figure 2: Effects of citral on colony growth of *A. flavus* 3.4408.

A. flavus decreased with the increase of citral concentration. When the concentration of citral was 10 μL, the colony growth rate was reduced by 15%. By observing the overall morphology of the colony, it was found that the addition of high concentration of citral could significantly reduce the diameter and growth of the colony, the spores in the colony were sparse, the spore production ability was decreased, and the physiological morphology was abnormal.

3.3. Inhibitory Effect of Citral on Growth and Toxicity of A. flavus 3.4408. The trend of growth and toxin production of *A. flavus* 3.4408 in the whole culture process with the change of culture days under the treatment with citral is shown in Figure 3. With the increase of the concentration of citral, the dry weight of *A. flavus* decreased, and the production of AFB1 also decreased. The concentration of citral was negatively correlated with the growth and toxin production. When the concentration of citral was 180 mg L^{-1}, the dry weight of mycelia was only 0.004 g, the inhibition rate of the growth was 98.79%, and the inhibition rate of toxin production was more than 99%. It can be concluded that citral can significantly inhibit the mycelium growth and the production of AFB1. According to the observation of mycelium pellet morphology of *A. flavus* (Figure 3), it can be found that large number of mycelia wound into pellets at low concentration, while, compared with the control group, mycelium pellets are smaller. When the concentration of citral reached 180 mg L^{-1}, the mycelia sparsely formed into strips. The results showed that citral had a significant inhibitory effect on mycelium biomass synthesis and also had a strong inhibitory effect on mycelium morphology. Citral can inhibit the biosynthesis of the secondary metabolite AFB1 by inhibiting the growth of *A. flavus*. The inhibition of citral on the growth and toxin metabolism of *A. flavus* had double effects, and there was an obvious concentration-effect relationship.

3.4. Effect of Citral on the Structure of A. flavus 3.4408. In this study, scanning electron microscopy (SEM) was used to investigate the effects of different concentration of citral on the structure of *A. flavus* 3.4408, reflecting the degree of inhibitory effect of citral on the growth of *A. flavus* 3.4408 (Figure 4). The spores of the control group with no treatment were in full shape and in large number. The spores of the experimental group had pores on the surface and collapsed at the concentration of 5 μL, and the spore size was smaller than that of the control group. With the increase of the concentration of citral, the destruction of spore structure was more serious—the spore head burst and the spore shrank. When no citral treated, the hypha surface was smooth and the thickness was even. Under the action of citral, mycelia were disorderly arranged, tightly wound, and twisted. Therefore, it can be speculated that citral will destroy the germination and growth of spores, rupture the cell membrane, cause collapse and necrosis of mycelium, and thus affect the normal physiological metabolism of *A. flavus*.

3.5. Identification and Toxicity Determination of A. flavus from Traditional Chinese Medicine. Herein, two strains were isolated from moldy Chinese medicinal materials. DNA of these two strains was extracted and sequenced, and the sequencing results were compared in GenBank. Then, the sequencing results were analyzed by RaxML V7.2.6 software, and the maximum likelihood (ML) phylogenetic tree is shown in Figure 5. The phylogenetic tree consists of six species with a total of 19 representative strains and an outgroup. The sequences obtained in this study were clustered in the same branch as *A. flavus*, and the support rate reached 100%. It was indicated that the isolated strains belong to *A. flavus*, named as ZK and ZX.

The toxicities test showed that there were significant individual differences among different strains of the same species, and the toxicities between *A. flavus* 3.4408 and ZK differed by three orders of magnitude. According to Chinese relevant provisions, the limit standard of AFB1 in Chinese medicinal materials is 5 μg kg^{-1}. In present study, the toxic amount of ZK is 3.01 μg kg^{-1}, with weak toxic capacity, while the toxic amount of ZX is 345 μg kg^{-1}. It was indicated that the *A. flavus* in moldy Chinese medicinal materials has certain toxic capacity, and its harm cannot be ignored.

3.6. Inhibitory Effect of Citral on ZK and ZX. According to the sensitivity test and spore counting test, the inhibitory effect of citral on ZK and ZX was researched. The sensitivity of citral on ZK and ZX was moderate (Figure 6). The inhibiting ability of citral on ZK was slightly stronger than ZX (Table 3). In high concentration, citral can completely inhibit the growth of ZK. The EC50 of ZK and ZX were 270.87 mg L^{-1} and 363.27 mg L^{-1}, respectively. Combined with the experiment of strain 3.4408, it was concluded that the inhibitory effect of citral on the *A. flavus* with different sources was different and citral can be used as an effective bacteriostatic material to inhibit *A. flavus* from Chinese medicinal materials.

4. Discussion

In recent years, consumers demand for effective and safe natural products to control food spoilage without chemical residues has increased to great extent. Essential oils are aromatic and volatile, produced as secondary metabolites of plants. Most essential oils play an important role in reducing food spoilage. In general, terpenoids are the main constituents of most essential oils, including monoterpenes, sesquiterpenes, and their oxygenated derivatives. Among them, monoterpenes are the active antimicrobial compounds of essential oils. Citral is one of the most important representatives of open chain monoterpenes accounting for 60–70% of the total *Litsea cubeba* oil. Besides, citronellal, α-terpineol, and linalool were accounted for the inhibition though their percentage in *Litsea cubeba* oil is low.

The sensitivity test can directly judge whether the compound is sensitive to the target bacteria, a way to screen sensitive compounds, and usually used as a preliminary study to find the inhibitory effects of essential oils on target

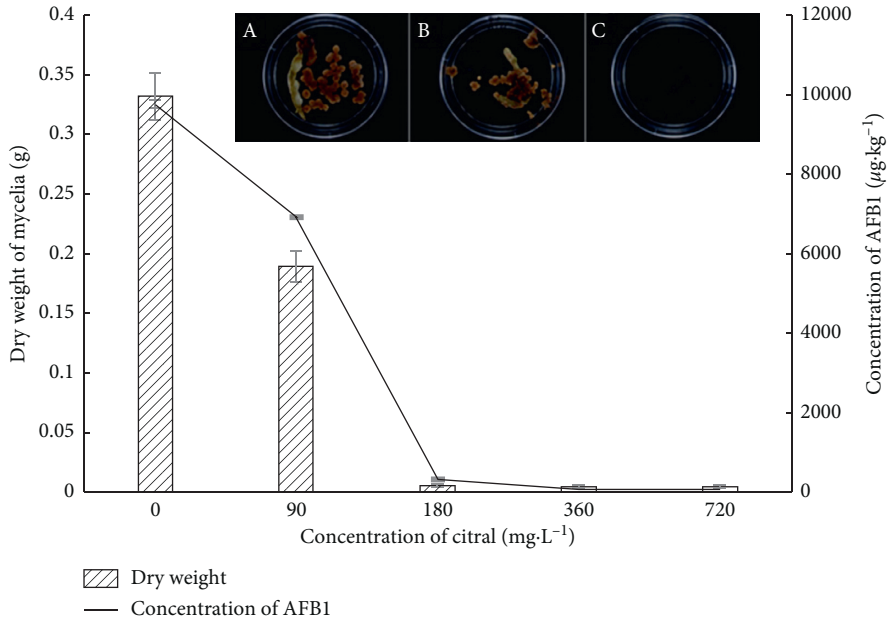

Figure 3: Inhibitory effect of citral on growth and toxicity of *A. flavus* 3.4408. ∗A: control (untreated); B: citral at 90 mg L^{-1}; C: citral at 180 mg L^{-1}.

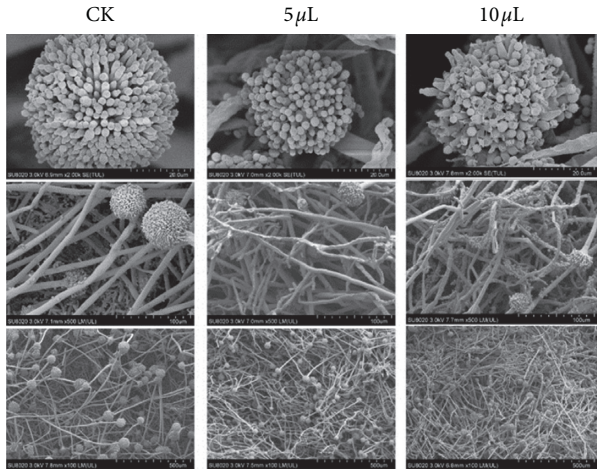

Figure 4: SEM of *A. flavus* 3.4408.

bacteria. However, it is necessary to calculate the half inhibition concentration or minimum inhibitory concentration in order to accurately judge the antibacterial ability potential of that particular compound. For instance, a study combined inhibition zone diameter with minimal inhibitory concentration to find antimicrobial activity of essential oils in the methanolic extracts of *Thymus nummularius* (Anzer tea) [22]. Therefore, these two methods were adopted to screen the compounds having strongest inhibitory ability for *A. flavus* 3.4408 for systematic bacteriostatic research and subsequent utilization. Herein, citral showed that it had best inhibition in the composition of *Litsea cubeba* oil.

Under both solid and liquid culture conditions, citral showed marked inhibition of *A. flavus* 3.4408. The citral mediated inhibition of *A. flavus* showed decline in growth in a time dependent fashion. Compared with the control, citral retarded the growth of hysteresis colony in the experimental group. Furthermore, citral had a concentration effect on the inhibition of *A. flavus*; i.e., the higher the concentration of citral, the smaller the colony diameter and the less bacterial biomass and toxin production are examined. Mycelial growth of *A. flavus* was reduced significantly at 150 mg L^{-1} concentration of *Zingiber officinale* essential oil [23], while 650 g mL^{-1} concentration of *Cinnamomum zeylanicum* essential oil showed MIC [24]. In the current study, the EC50 of citral on fungal spores was 163.65 mg L^{-1}, and 180 mg L^{-1} concentration significantly inhibited the production of mycelia and toxins as comparable to the inhibition mediated by citral for *A. flavus*.

The results also showed that citral can potentially inhibit dry mycelium weight and the AFB1 synthesis of *A. flavus* 3.4408. Literature showed a direct correlation between

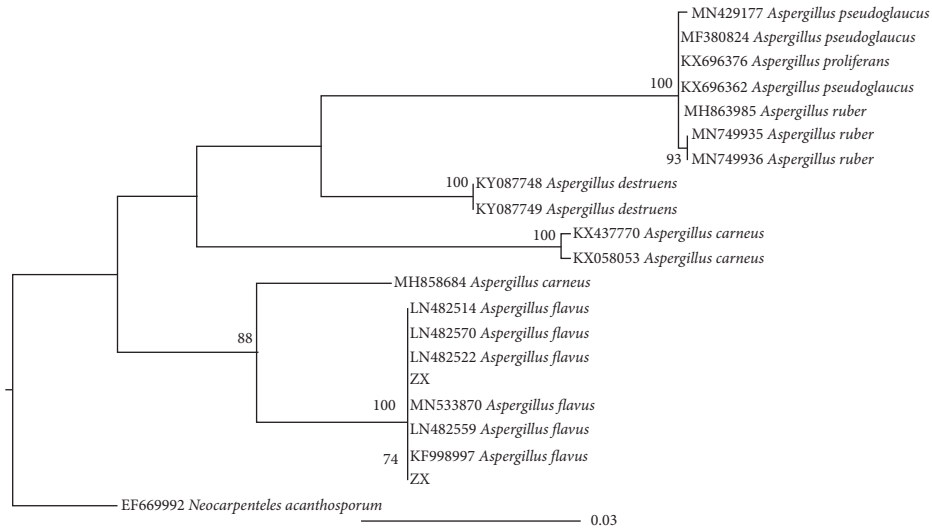

Figure 5: Phylogenetic tree shows the origin of two strains (ZK and ZX) of the fungus.

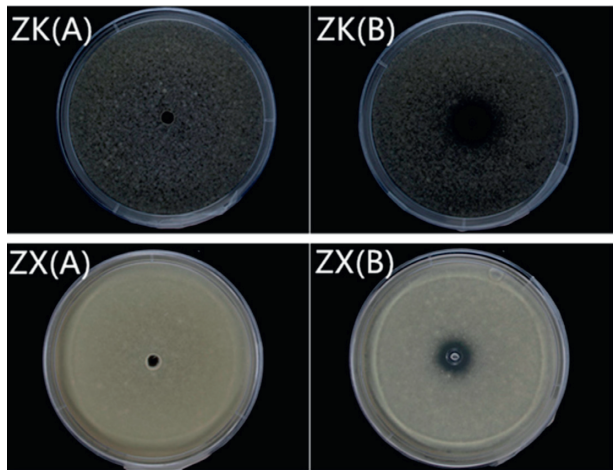

Figure 6: The sensitivity of citral on ZK and ZX. A: control (untreated); B: the experimental group (150 mg L^{-1}).

Table 3: The toxicity equation of citral on ZK and ZX.

	Concentration (mg·L^{-1})					Toxicity equation	EC50 (mg L^{-1})
	90	180	360	720	1440		
ZK	13.89% ± 7.0%	16.29% ± 3.89%	30.87% ± 10.11%	63.12% ± 10.22%	100%	$y = 4.4807x - 5.9004$	270.87
ZX	24.94% ± 9.33%	32.57% ± 9.17%	52.42% ± 2.68%	58.52% ± 6.84%	79.64 ± 2.56%	$y = 1.2217x + 1.8721$	363.27

fungal growth and AFB1 production [25, 26]. However, the inhibition of AFB1 production cannot be completely attributed to the insufficient fungal growth, but instead it can also be linked with the change in microstructure with following citral treatment. It is also elucidated that, citral combined with aflatoxin is a good strategy for complete removal of the fungus. Hence, determining the AFB1 suppression mechanism requires further investigation.

Most of the studies used type strains to carry out scientific research, while the current study explored the potential of citral as a mildew preventive agent for medicinal materials. It is not convincing to use only the type strains as the research object, but various strains of *A. flavus* isolated from moldy Chinese medicinal materials could be ideal strains. Based on the results, citral appeared to inhibit *A. flavus* from moldy Chinese medicinal materials.

5. Conclusions

The inhibitory effect of *Litsea cubeba* oil and its active components on *A. flavus* 3.4408 showed that citral had the best inhibitory effect on growth. The inhibition of bacterial growth rate, biomass, and AFB1 synthesis showed good bacteriostatic ability of citral to *A. flavus* strains (ZK and ZX) isolated from moldy Chinese medicinal materials. It should be encouraged to use citral as mildew preventive agent for

Chinese medicinal materials. Furthermore, the inhibition mechanism on *A. flavus* should be deeply clarified to provide more reliable theoretical basis.

Data Availability

All data and analyses are included as figures and tables within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Guorong Fan contributed to this work.

Acknowledgments

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Review Article

Antimicrobial Activity of Pomegranate Peel and Its Applications on Food Preservation

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Pomegranate (*Punica granatum* L.) fruit is being cultivated since the civilization is known, and its production and consumption have been increased since the last century due to the scientific confirmation of its health benefits. Pomegranate fruits, fruit juice, its seeds, and peels are known to have higher contents of bioactive compounds, viz., phenolic acids, flavonoids, and hydrolysable tannins. The peels of pomegranate fruits are the major by-products produced during food processing of pomegranate enriched in antioxidants and broad-spectrum antimicrobial agents and can prevent food deterioration even. This health potential of pomegranate is known to vary significantly upon the varieties, growing conditions, cultivation practices, stages of the development, and the extraction methods. Herein, the biochemical composition of the pomegranate peel extract (PPE), its efficacy in food preservation, and antimicrobial activities are discussed to provide a comprehensive guide for farmers, food processing, and storage sectors and academia.

1. Introduction

Pomegranate (*Punica granatum* L.) plants are among the first cultivated plants by humanity; however, its consumption had been limited most commonly as a result of the hassle of extracting the juicy arils [1]. Due to the increasing number of scientific studies about its health benefits, production and consumption of pomegranate fruits have been increasing since the beginning of the 21st century. Pomegranate fruits are consumed as both fresh and processed mainly in the forms of juice, oil, wine, and jams. Both the fruits and its peel are known to have high levels of numerous phytochemicals, including phenolic acids, flavonoids, and tannins. This diverse characteristic of phytochemicals is thought to be responsible for its high antioxidant potential and health benefits [2]. During processing, a considerable amount of by-products are developed from peels and is known to have high contents of hydrolysable tannins (HTs) [3]. Recently, by-products of pomegranates, especially pomegranate peel extract (PPE), have been increasing attention due to its scientifically confirmed therapeutic properties such as antioxidant, antimicrobial, anticancer, antiulcer, and anti-inflammatory activities [4, 5]. Numerous scientific studies have suggested that PPE exhibits excellent antimicrobial activity against several foodborne pathogens and improves the postharvest storability of food products [6, 7]. This paper will describe and discuss the recent advancements about the biochemical composition, antimicrobial potential, and food preservation characteristics of PPEs.

2. Biochemical Composition of Pomegranate Peel

Pomegranate peels have high levels of numerous phytochemicals [2]. It has been reported that PPE is high in bioactive compounds, mainly phenolic acids, flavonoids, and hydrolysable tannins [8, 9]. The primary phenolic acids

identified from PPEs are ellagic acid, gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic acid, vanillic acid, p-coumaric acid, and cinnamic acid [10–14]. Phenolic acids concentration significantly varies among varieties and is highly depending upon the geographical location, climatic conditions, and cultivation practices [9, 13]. One of the main parameters defining the concentration of the phenolic acids was noted as the peel colour where the varieties with dark red colour reported to have higher phenolic acids concentration than the light-coloured varieties [15]. Additional to phenolic acids, PPEs are an excellent source of flavonoids. Flavonoid content and composition are also known to vary significantly among varieties and growing conditions; however, it was also noted that the fruit developmental stage influence the flavonoid content and composition [6, 16, 17]. Besides, PPEs are reported to have rich sources of tannins. The reported tannins are ellagitannins, punicalagin, granatins, punicalin, pedunculagin, castalagin, corilagin, gallagyldilactone, and tellimagrandin [18, 19]. Almost 49 compounds, the majority of which were flavonoids, phenolic acids, and tannins [9, 19–26] isolated from pomegranate peels, are summarized in Figure 1.

The antioxidant activity of the PPEs is attributed to the phenolic acids, flavonoids, and tannins. Among these, ellagitannins are noted to be most responsible for the antioxidant activity of the pomegranate peels [9]. It was also stated that both concentrations of phytochemicals and antioxidant activity are highly dependent upon the solvents used for the peel extraction. Previous studies also suggested that the methanolic extracts of the pomegranate peels exhibit higher antioxidant activity as compared with other extractions methods [27]. The concentrations of phenolic acids, flavonoids, and tannins in PPEs mainly depend on the extraction method. For example, Orak et al. [28] reported the highest concentrations of tannins identified from methanol extracts, as compared with water and ethanol extracts. Acetone extracts of pomegranate peels were also noted to have higher antioxidant activity than the water and ethanol extracts [19].

3. The Extract Process of Pomegranate Peel

One of the most widely used methods for pomegranate peel extraction is the methanol extraction method reported by Dahham et al. [29]. In this method, first of all, the fine peel powders are obtained by an electric blender and oven-dried at 40°C for 24 h. Then, the powders are sieved through a 24-mesh, and 10 g of powder sample is extracted with 250 ml of 80% methanol at room temperature (~25°C) for 24 h. The final extract is then filtered and used. If the aqueous extract is needed for use, the powdered sample (10 g) is extracted with 100 ml of distilled water and used. The drying temperature and duration was reported as 50°C for 48 h in some other studies [11].

Water, ethanol, and acetone are the other solvents reported to be used instead of methanol for the extraction of the pomegranate peel [19, 28]. Most of the published literature about the different extraction methods/solvents recommended that the methanol extraction method is better than others in terms of the abundance of phenolic compounds and increase antioxidant activity [28, 30–32].

The extraction method is reported to have a strong influence on the biochemical composition of PPEs. The conventional techniques generally need high amounts of solvents and results with low extraction yields. Moreover, the use of high temperature is reported to result in degradation in the extracts [33]. Recently, high-pressure extraction is observed and noted as a practical methodology, which results with no detrimental effects on bioactive compounds. Additionally, to be a green technology, the high-pressure extraction is faster and has a high yield [34].

Another important extraction method is the enzymatic extraction. It was reported to assist in the extraction of bioactive compounds. The mechanism behind the enzymatic extraction involves some cell wall degrading enzymes (i.e., pectinases). These enzymes break down the cell wall and improve the extraction of the bioactive compounds [35, 36].

4. Antimicrobial Activity of Pomegranate Peel

Pomegranate has a broad spectrum of antimicrobial effects, which has an apparent inhibitory effect against Gram-negative, Gram-positive bacteria (Table 1), fungi, and mould (Table 2). However, different extracts from different parts of pomegranate have various antimicrobial activities. The study of many scholars showed that the antimicrobial activity of PPE was more potent than other parts, and the antimicrobial activity of PPE was related to the total flavonoids and tannins content. PPE is well known for its antimicrobial activity against bacterial and fungal pathogens [29, 37, 38]. However, the number of studies which investigated the effects of PPE against plant pathogenic bacteria and fungi is limited [11, 32, 39, 40]. PPE was noted to have antibacterial effects on different foodborne pathogens including *Escherichia coli*, *Fusarium sambucinum*, *Penicillium italicum,* and *Bacillus subtilis* [11, 31, 38, 41]. In one of these comprehensive studies, the antimicrobial activity of extracts derived from different parts of pomegranates (peel, seeds, juice, and whole fruits) was tested on seven bacteria (*Bacillus coagulans*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*). The maximum inhibitory of all bacteria was noted from the peel extracts of pomegranates [29]. Pomegranate peel was also tested as an incorporation agent into bio-based films and was found to improve the antibacterial activity of materials. In one of these studies, Ali et al. [42] reported that PPE inhibited the growth of *S. aureus* (Gram-positive) and *Salmonella* (Gram-negative). As discussed in Section 3, the extraction method can significantly influence the antimicrobial activity of pomegranate peels. In a recent study, antimicrobial effects of high pressure (300 and 600 MPa) of the pomegranate peels were tested by [13]. In this study, the maximum antioxidant activity and phenolic content were observed at the extraction of 300 MPa; thus, the higher antimicrobial activity against pathogenic bacteria was noted from 300 MPa. The ethyl acetate extract of pomegranate peel was previously reported to include high concentrations of tannins which are very active against *Staphylococcus aureus*

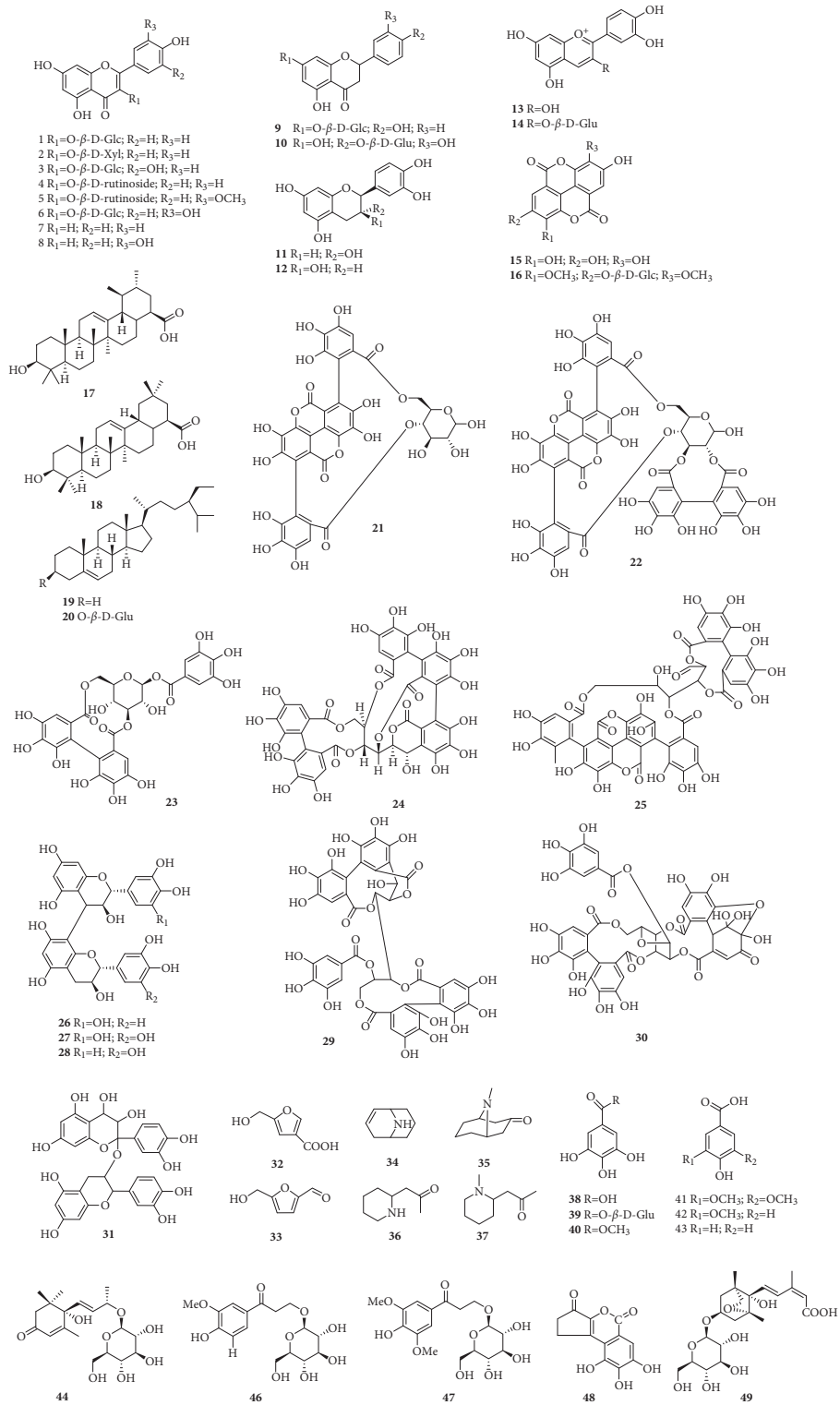

Figure 1: Chemical structures of reported compounds from pomegranate peels.

strains [43]. Water extract of pomegranate peel was also reported to control Gram-positive significantly and Gram-negative bacteria including *E. coli*, *B. subtilis*, *Enterobacter aerogenes*, *Serratia marcescens*, *Brucella spp*., *Saccharomyces cerevisiae,* and *Rhodotorula glutinis* [44].

In a different study, the effects of PPEs for controlling the growth and development of *Fusarium sambucinum* were tested in in vitro as curative and preventive. The researchers noted that the PPE (20 mg/ml) exhibited complete inhibition of spore germination and 75.5%

Table 1: Antibacterial effects of pomegranate peel against different pathogens.

Bacteria	Peel preparation and dose	Mechanism of action	Reference
Eight different food contaminants/pathogenic bacteria	Under high pressure at 300 MPa	Due to high antioxidant activity and high total phenolic content	[13]
B. coagulans, *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae,* and *P. aeruginosa*	10 g sample (sample = 10 g peel extracted with 250 ml 80% methanol) extracted with 100 mL water	Estimated to be a result of high phytocompounds including phenols, tannins, and flavonoids.	[29]
S. aureus and *Salmonella*	2, 4, 6, 8, 10, 12, and 14% PPE with 20% polyethylene glycol	Inhibition of films against targeted microorganisms	[42]
S. aureus	The ethyl acetate extract of pomegranate peel	Due to the high concentrations of tannins	[43]
Gram-positive and Gram-negative bacteria including *E. coli*, *B. subtilis*, *E. aerogenes*, *S. marcescens*, *Brucella* spp., *S. cerevisiae,* and *R. glutinis*.	Water extracts of pomegranate peels (50/100 w/v)	Due to high tannins content	[44]

Table 2: Antifungal effects of pomegranate peel against different pathogens.

Fungi	Peel preparation and dose	Mechanism of action	Reference
F. sambucinum	The methanol extract of pomegranate peels (20 mg·ml^{-1})	Due to the high contents of phenolic compounds	[11]
P. digitatum and *Saccharomyces cerevisiae*.	0.061–0.304 g dry methanolic extract mL^{-1}	Inhibit cellular receptors of pathogens	[32]
Brown rot (caused by *Monilinia laxa* and *M. fructigena*)	Aqueous PPEs (9.93 and 12.84 mg·ml^{-1})	Due to the high total phenolic and flavonoid contents	[45]
B. cinerea, P. digitatum, and *P. expansum*	20 h of incubation with PPE (1.2 and 12 g·L^{-1})	Due to the high concentrations of ellagitannins	[46]
F. oxysporum f. sp. *lycopersici*	Water extract (80%), methanol extract (40%), and ethanol extract (20%) of pomegranate peel	Due to the high levels of total phenol and punicalagin contents.	[47]
P. digitatum	Incorporation of 0.361 g water PPE into locust bean gum and chitosan coatings	Due to the high contents of phenolic compounds	[48]

inhibition on mycelia growth of fungi [11]. In a recent study, El Khetabi et al. [45] studied the in vitro and in vivo effects of aqueous PPE on the brown rot (caused by *Monilinia laxa* and *M. fructigena*). They reported an inhibition varying from 76.65% to 90% on the control of mycelia growth. Intense fungicidal activity of PPE was then reported against *Botrytis cinerea*, *Penicillium digitatum,* and *Penicillium expansum* by Nicosia et al. [46]. 20 h of incubation with PPE resulted with almost complete inhibition of all fungal spores at lemons and grapefruits. A recent study investigated the antifungal activity of 21 different pomegranate genotypes [47]. Researchers noted that all of the tested genotypes have varying antifungal activities, against *Fusarium oxysporum* f. sp. *lycopersici*, where some of them completely inhibit the fungus, and some others had deficient inhibitory activity. The two different extracts of pomegranate peel (water and methanol) were incorporated with chitosan and locust bean gum for controlling *P. digitatum* at orange fruits. Results suggested that the addition of 0.361 g water PPE into both locust bean gum and chitosan coatings significantly reduce the *P. digitatum* by 28% and 49%, respectively [48]. Phenolic profile and antimicrobial activity of "Gabsi" PPEs were studied by Kharchoufi et al. [32]. Similar to the previous studies of the researchers, the water and methanol solvents were tested separately. Researchers noted different results than their other studies and recommended that the methanol extraction is more effective against the *P. digitatum* and *Saccharomyces cerevisiae*.

There are still significant obstacles for the industrial use of PPEs to control postharvest pathogens. This limitation is due to the unclear mode of action of PPE, high costs, lack of curative effect, significant variation among varieties, climatic conditions, cultivation practices, and extraction methods and limited range of activity against different fungal pathogens [9, 49].

5. Biochemical Changes and Food Preservation of Pomegranate Peel

PPE is a valuable by-product for the food preservation industry. As discussed previously, PPE is a rich source of bioactive compounds, including tannins consisting of ellagic acid and gallic acid [42, 50, 51]. Most of the bioactive compounds, which are abundant in the PPE, were previously tested as natural additives for improving the preservation quality of food [52]. PPE was yet tested alone or in combination with edible films and coatings for food preservation. Renewable, bio-based, environment friendly active packaging systems, which are usually composed of biopolymers such as proteins, lipids, and polysaccharides [53], have been extensively used in food packaging since the

beginning of the 21st century [54]. Legume seeds as a good source of plant proteins (25–28%) have a good potential as a bio-based film [55].

Moreover, incorporation of natural compounds (with high contents of antioxidant activity, phenolic compounds, and essential oils) into bio-based films is known to improve the activity of edible coatings and enhance the storage duration of food products [56]. Among these, fruit peels constitute an essential part, which is a rich source of bioactive compounds. PPEs have also been widely used in the formulation of bio-based edible coatings/films [57]. The PPE was also noted to reduce the water vapour permeability of chitosan-based film material and its antimicrobial activity [58]. Another recent study showed that the incorporation of different concentrations of PPE with mung bean protein films provides a biofunctional edible film for packaging of food products [59]. The incorporation of PPE was reported to give flexibility to the films, increase the thickness and water vapour permeability, and decreases the moisture content. It was determined that PPE retains its semicrystalline structure in bio-based edible films and improves the efficacy of the material [42]. In a similar study, incorporation of PPE in chitosan was tested for food packaging [60]. The addition of PPE to active polyvinyl alcohol (PVA) composite film also resulted in high antioxidant activity and antibacterial ability [61].

The incorporation of PPE (1% w/v) into chitosan (1% w/v) and alginate (2% w/v) coatings was then reported to improve the postharvest storability of "Allahabad safeda" guava fruits. Results suggested that the edible coatings reduced the respiration rate, protected ascorbic acid content, total phenolics, total flavonoids content, and antioxidant activity, and maintained the overall fruit quality [62]. The phenolics in foods capture free radicals, produced during oxidative stress, and prevent the deterioration of the foods [63]. Several studies with other edible coatings confirm that the edible coatings reduce the respiration rates and help to maintain the postharvest quality of fresh fruits [64, 65]. Reduction in respiration rate results with a decrease in the enzyme activity and thereby resulting in a reduction of ascorbic acid oxidation [66] and improves the postharvest storability of foods. Higher respiration rate results in the breakdown of total phenols, and this accelerates the ageing process. Thus, the reduction of the respiration rate by the edible coatings enriched with PPE results with a high amount of phenolic compounds [31] and improved storability of the foods.

Rather than the direct influence on the pathogens, PPEs also induce plant resistant to pathogens. In one of these studies, PPE was reported to cause a transcriptomic response at orange fruits. It was noted that PPE upregulates 273 significant genes and downregulates eight genes. Changes in the gene expressions were noted to enrich antibiotic biosynthesis and induce defence pathways [67].

6. Prospective

Pomegranate peel is the waste produced in the process of pomegranate food processing, accounting for 20–30% of the total weight of pomegranate. The high antioxidant activity, inhibition of lipid peroxidation, and broad-spectrum antimicrobial efficiency of pomegranate peel play an intrinsic quality foundation for its development as a food preservative. To make pomegranate peel more widely used in food preservation, the following aspects need to be further studied and explored:

(1) Study on active antimicrobial components of pomegranate peel. Pomegranate peel composition is complicated. Although the chemical composition of pomegranate peel was well studied, the number of specific studies about the mode of action is relatively few. Thus, the direct causes of antimicrobial activities of pomegranate peel are still unclear, and there are other antimicrobial substances in pomegranate peel, which still need further research.

(2) Study on the antibacterial mechanism of pomegranate peel. Pomegranate peel has an excellent bacteriostatic effect, but the mechanism of its bacteriostatic effect has not been studied intensely. Furthermore, research on the mechanism of bacteriostatic impact will be helpful to promote the application of pomegranate peel in the field of food preservation.

(3) Study on the compatibility of pomegranate peel and other chemical food preservatives. Food preservatives from different sources have a synergistic preservative effect. Studying the optimization of the formula of pomegranate peel and other chemical preservatives can enhance the preservative effect and reduce the amount of chemical preservative.

Data Availability

The data used to support the findings of this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Synthesis and Antifungal Activities of Cinnamaldehyde Derivatives against *Penicillium digitatum* Causing Citrus Green Mold

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Penicillium digitatum (green mold) is pathogenic fungi and causes citrus fruit postharvest rotting that leads to huge economic losses across the world. The current study was aimed to develop a new derivative of cinnamaldehyde (4-methoxycinnamaldehyde) through the cross-hydroxyaldehyde condensation method with benzaldehyde substituted by a benzene ring under the catalysis of alkaline reagent and, moreover, to test their antifungal potential against *P. digitatum*, the major citrus fruit rotting fungi. Multiple derivatives of cinnamaldehyde *viz.* 4-nitro CA, 4-chloro CA, 4-bromo CA, 4-methyl CA, 4-methoxy CA, and 2,4-dimethoxy CA were synthesized in the current study whereas the 4-methoxy CA showed highest antifungal actions for citrus fruit postharvest rotting fungi *P. digitatum*. Moreover, 4-methoxy CA was found to reduce the spore germination and growth by damaging the fungal cell membrane, as well as declined the levels of reducing sugars.

1. Introduction

Many plant pathogenic fungal strains are known to cause huge economic losses because of the reduction in yield and production throughout the world. Green mold (*Penicillium digitatum*) is an important pathogen causing postharvest losses at citrus fruits. Citrus fruit is a commercial crop grown across the globe and is highly consumed due to its high nutritional value and health benefits [1]. During postharvest storage, multiple biotic and abiotic factors may lead to the decay at citrus fruits mainly caused by the green mold (*P. digitatum*) causing approximately 90% of postharvest yield loss [2–4]. At present, various physical and chemical treatment methods are in practice to control the postharvest green mold. Such treatment methods include the application of certain synthetic and natural fungicides, where the synthetic fungicides are considered as the standard treatment method for controlling citrus postharvest green mold but have certain environmental and health issues [5–7].

Due to increasing health concerns, synthetic fungicides are being replaced gradually by the natural phytochemicals bearing strong antifungal potential in recent years. Since the last decade, the researchers are focusing on the use of some alternatives of the synthetic fungicides by natural plants or

their phytochemical derivatives, such as cinnamaldehyde for controlling of citrus postharvest diseases [8–10]. Cinnamaldehyde along with its various derivatives has been well classified as potential antifungal agents [11, 12]. Furthermore, excessive use of synthetic chemicals for controlling fungal born pathologies is truly a challenging job, because of the increase in the fungi resistance to chemicals, hence leading to explore novel antifungal chemicals. Cinnamaldehyde is studied extensively and reported to inhibit the growth of various pathologic bacterial strains [13, 14] and many fungi [15] through acting on the ATPase activities, biosynthesis on the cell walls, and modulation in the membranous structures [16–18].

Cinnamaldehyde (CA) is a major component in the cinnamon essential oils extracted from the cinnamon tree bark [19]. Different from many other related natural compounds, cinnamaldehyde has been used as a potential antifungal agent, but its volatile nature and pungent smell limit its application. Thus, this led the scientists to modify cinnamaldehyde into stable but acceptable derivatives [20–22]. Cinnamaldehyde was complexed with Schiff bases (N,N,-bis(*p*-methoxycinnamaldehyde) ethylenediamine) and found that the resulting complex has better biological activities [23]. Moreover, α-substituted cinnamaldehyde derivatives (α-bromocinnamaldehyde, α-chlorocinnamaldehyde, and α-methylcinnamaldehyde) have strong antityrosinase activity [24]. In another study, α-bromocinnamaldehyde showed antifungal properties against many of the household items and gadgets was reported to disrupt the metabolic activities of the fungal cells [25]. 2′-Benzoyloxycinnamaldehyde has known to affect the growth of fungal hyphae by reducing the expression of chitin synthases of many of the human pathogenic fungi [26], where similar reduction in fungal growth has also seen by two more similar derivatives (2-chloro- and 2-bromocinnamaldehyde). A variety of amino acid (AA) composed derivatives of CA were successfully developed and found to have potential antifungal agents, particularly for *Saccharomyces cerevisiae* [27]. Different strains of *Candida* were also tested for the effective antifungal potential of the o-methoxy CA, and a marked and clear reduction in the growth of various *Candida* strains was mainly targeting to membranous H^+-ATPase by the CA [28]. Moreover, α-bromo CA and p-nitro-α-bromo CA are quiet effective against the proliferation of *Candida albicans* and *Trichophyton mentagrophytes* [29]. N-(cinnamyl) chitosan derivatives were also reported to have antifungal potential against many fungal strains viz. *Botrytis cinerea*, *Alternaria alternata*, *Fusarium oxysporum* (*F. oxysporum*), *F. solani*, *Pythium debaryanum*, *Botryodiplodia theobromae*, and *Phytophthora infestans* [30].

Keeping in view the diverse physiological effects of cinnamaldehyde and its derivatives, herein we attempted to develop a new derivative of cinnamaldehyde (4-methoxycinnamaldehyde) and to test its antifungal potential against *Penicillium digitatum*, the major citrus fruit rotting fungi. The current study also aimed to understand and elucidate the basic mechanism of action of 4-methoxy CA on the growth of the green mold, germination of spores, and permeability of fungal cell membranes.

2. Materials and Methods

2.1. The Pathogenic Fungal Strain and Culture Medium. The isolates of *Penicillium digitatum* inoculum were taken from some infected citrus fruits with typical green-mold signs, cultured on a potato dextrose agar (PDA) medium at 27°C for seven days and preserved (4°C). The collection of mature spores was done by washing PDA surface using autoclaved distilled H_2O, and then spore suspensions were passaged from a sterilized cotton ball in a funnel to check the hyphae. The spore suspension of *P. digitatum* was counted in a Countess-II FL automatic cell counter (Thermo Fisher Scientific, Waltham, MA) and set at 1×10^6 CFU/mL using autoclaved distilled H_2O.

2.2. In Vitro Evaluation of Antifungal Activity

2.2.1. Disc Diffusion Assay. The modified method of Bauer–Kirby disk tests was used for measuring zones of the antifungal activities of cinnamaldehyde and its derivatives [31]. Petri dishes (diameter, 90 mm) were prepared with a PDA medium (about 15 mL) and surface inoculated with the optimal concentration of spore suspensions in sterile water. The sterile Oxford cup (diameter, 8 mm) was impregnated with 200 μL of six cinnamaldehyde derivatives. The diameters of inhibitory zones around the Oxford cups were measured in mm after 72 h of culture at 27°C under darkness. The six cinnamaldehyde derivatives were judged to be a potential antifungal agent when the diameter of inhibitory zone was larger than 8 mm.

2.2.2. Measurement of Minimal Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). The MIC and MFC of 4-methoxy CA on the mycelial growth of the tested fungal strains were determined using the agar dilution method described previously [32]. Different concentrations of 4-methoxy CA were mixed with PDA in a proportion of 1 : 9 for obtaining the final concentrations of 0, 15.6, 31.3, 62.5, 125, 250, and 500 μg/mL. The 6 mm-diameter mycelial disks cut from a 7-day-old culture of the tested fungal strains were placed in the centre of each Petri dish and incubated at 27°C. The MIC and MFC were defined as the lowest concentration of 4-methoxy CA that 100% inhibited the mycelial growth of *P. digitatum* after 2nd d and 6th d of incubation at 27°C, respectively [33].

2.3. Inhibitory Effect of 4-Methoxy CA on Spore Germination. Serial concentrations (15.6–250 μg/mL) of 4-methoxy CA were tested for their activity against spore germination inhibitory (SGI), with the absence of 4-methoxy CA serving as a control. The PDB medium amended with 4-methoxy CA was transferred onto a concave slide. 20 μL of spore suspension (1×10^5 spores·mL^{-1}) was added. All the inoculated slides were placed on moist filter paper on Petri plates at 27°C for 12 h. The spore germination was observed using a reversed biological microscope (Olympus CKX53, Japan). The spores were defined as germinated if the germ tube

length was equal or exceeded that of the spore [32]. The inhibition of spore germination was calculated using the following formula:

$$\text{Spore germination inhibition (SGI \%)} = \frac{\text{Gc} - \text{Gt}}{\text{Gc}} \times 100, \quad (1)$$

where Gc and Gt are the mean number of germinated spores of control and treated slides, respectively. Four replicates were used per treatment, and the experiment was carried out with two separate times.

2.4. Determination of Membrane Permeability. The determination of extracellular conductivity was carried out using a ST3100c/F electrical conductivity meter (Ohaus Co., Ltd., New Jersey, America). After 48 h of shake inoculating in PDB at 27°C, 5.0 g of the healthy mycelia of *P. digitatum* was resuspended in 100 mL PDB with the 4-methoxy CA stock solution to the final concentrations of 1/2 MIC, MIC, 2 MIC, and MFC. The extracellular electric conductivity was determined at 0, 30, 60, 90, and 120 min of the 4-methoxy CA-treated and control PDB, and expressed as the amount of extracellular conductivity (μs/cm).

2.5. Assay for Intracellular Reducing Sugars. The reducing sugar content of *P. digitatum* mycelia, treated with 4-methoxy CA using a range of concentrations (0, MIC, 2 MIC, and MFC), was examined through the anthrone colorimetric method [32] with certain minor changes. About 0.5 g (wet weight) of *P. digitatum* mycelia of 4-methoxy CA-treated and control PDB groups was collected, and homogenization was done in 8.0 mL of dH_2O; extracts were taken out through boiling H_2O bath for quarter of an hour. Afterwards, these isolated mycelial materials were quickly cooled by placing at 25°C for 10 min. Different dilutions were prepared using dH_2O, the volumes of these suspensions were raised to 100 mL, and 1.0 mL of 10% (v/v) lead acetate was mixed in it. Keeping this solution for about 15 min at room temperature, this suspension was filtered by a Buchner funnel after mixing 0.1 g of crystal violet oxalate in it. Then, 2 mL of the filtrate was taken out and heated for 5 min by placing it in boiling water bath after mixing 0.5 mL of anthrone reagent (BR 9012-76-4, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and 5 mL of H_2SO_4 in it, and it was incubated again for further 10 min in boiling water bath. After cooling this by placing on ice for 10–15 min, the absorbances were measured at 620 nm to tabulate the levels of reducing sugar from a standard curve using glucose as a standard.

2.6. Statistical Analysis. The data obtained were analyzed by using SPSS 17.0 version (SPSS Inc., USA). The Student's *t*-test was applied to analyze the MGI assay to examine actual levels of reducing sugars. The levels of significantly different values were gone through one-way analysis of variance (ANOVA), and the p value was set at 5%.

3. Results and Discussion

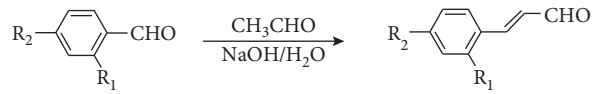
3.1. Synthesis of Cinnamaldehyde (CA) Derivatives. Substituted benzaldehyde, 40% acetaldehyde solution and sodium hydroxide solution were added to the reaction bottle and heated to reflux (Figure 1). The molar ratio of feeding is substituted benzoic aldehyde: acetaldehyde: sodium hydroxide = 1 : 1 : 1. After the reaction is completed (TLC tracking the reaction), we cool it to room temperature, add ethyl acetate extraction, combine organic layer, wash with brine, dry anhydrous magnesium sulfate, filter, reduce pressure to recover ethyl acetate solvent, and get crude products. Crude products for column chromatography purification to obtain cinnamaldehyde derivatives a–f and ^{1}H-NMR identified its constructures (Table 1).

3.2. Antifungal Activity of Cinnamaldehyde (CA) and Its Derivatives. The *in vitro* antifungal activities of CA along with its six derivatives (a–f) were examined against *P. digitatum*, and their antifungal potential was studied qualitatively by either presence or the absence, and the inhibitory zone sizes. As is shown in Figure 2, the results revealed that 4-methoxy CA had the maximum inhibitory zone (32.25 ± 0.35 mm) and the highest antifungal activity, followed by CA (31.40 ± 0.57 mm), 2,4-dimethoxy CA (29.40 ± 0.57 mm), 4-methyl CA (21.40 ± 0.57 mm), 4-chloro CA (20.50 ± 0.71 mm), 4-bromo CA (17.50 ± 0.71 mm), and 4-nitro CA (16.80 ± 0.29 mm).

Among the six CA derivatives, only 4-methoxy CA exhibited more pronounced antifungal activity against *P. digitatum* than CA (Figure 2), and the data in Table 2 ulteriorly confirmed the above result. This finding indicated that 4-methoxy CA could represent a new antifungal agent for inhibiting *P. digitatum* growth. Results of the current study are in agreement with the reports of QuYang et al. [34] who noted that the cinnamaldehyde has strong antifungal activity against *P. digitatum*. In a different study, it was also noted that the incorporation of *trans*-cinnamaldehyde with poly(lactic acid) and poly(butylene adipate-co-terephthalate) provides better performance as an edible film [35]. Overall, results support the idea suggested by Moosa et al. [36] where the biomaterials were reported as high potent against green and blue mold of citrus fruits.

The MIC and MFC examinations are being applied as one of the most important indexes to access the inhibitory efficiency of antifungal chemicals. The observation was made for *P. digitatum* of mycelial growth using the PDA medium with 4-methoxy CA treatments at 0, 15.6, 31.3, 62.5, 125, 250, and 500 μg/mL during the incubation period at 27°C, and 4-methoxy CA treatments at the concentration of 31.3 μg/mL and 125 μg/mL showed 100% inhibition of *P. digitatum* growth on 2nd and 6th days of incubation, respectively. So, 31.3 μg/mL and 125 μg/mL were the MIC and MFC of 4-methoxy CA which inhibit against *P. digitatum*.

3.3. Effect of 4-Methoxy CA on Spore Germination. 4-Methoxy CA significantly ($p < 0.05$) inhibited the spore germination, and the inhibition exerted a dose-dependent



a: $R_1 = H$; $R_2 = NO_2$
b: $R_1 = H$; $R_2 = Cl$
c: $R_1 = H$; $R_2 = Br$
d: $R_1 = H$; $R_2 = CH_3$
e: $R_1 = H$; $R_2 = OCH_3$
f: $R_1 = OCH_3$; $R_2 = OCH_3$

Figure 1: Synthesis of cinnamaldehyde (CA) derivatives.

Table 1: ^{1}H-NMR (400 MHz, $CDCl_3$) data of cinnamaldehyde (CA) derivatives.

Compounds	Name	Yield (%)	^{1}H-NMR (400 MHz, $CDCl_3$), δ
a	4-Nitro CA	88.12	6.81 (dd, J = 8.0, 16.0 Hz, 1H, CHCHO), 7.53 (d, J = 16.0 Hz, 1H, PhCH), 7.72–8.52 (m, 4H, ArH), 9.68 (d, J = 8.0, 1H, CHO).
b	4-Chloro CA	87.32	6.67 (dd, J = 7.6, 16.0 Hz, 1H, CHCHO), 7.39–7.49 (m, 5H, PhCH), 9.68 (d, J = 7.6, 1H, CHO).
c	4-Bromo CA	81.25	6.68 (dd, J = 7.6, 16.0 Hz, 1H, CHCHO), 7.40–7.56 (m, 5H, PhCH), 9.69 (d, J = 7.6, 1H, CHO).
d	4-Methyl CA	82.35	2.38 (s, 3H, CH_3), 6.69 (dd, J = 7.6, 16.0 Hz, 1H, CHCHO), 7.23–7.48 (m, 5H, PhCH), 9.68 (d, J = 7.6, 1H, CHO).
e	4-Methoxy CA	86.65	3.88 (s, 3H, OCH_3), 6.60 (dd, J = 7.6, 16.0 Hz, 1H, CHCHO), 6.93 (d, J = 6.4, 2H, ArH), 7.42 (d, J = 7.6, 1H, PhCH), 7.52 (d, J = 8.8, 2H, ArH), 9.64 (d, J = 7.6, 1H, CHO).
f	2,4-Dimethoxy CA	81.23	3.91 (s, 6H, OCH_3), 6.60 (dd, J = 7.6, 16.0 Hz, 1H, CHCHO), 6.85–7.07 (m, 3H, ArH), 7.40 (d, J = 16.0, 1H, PhCH), 9.64 (d, J = 7.6, 1H, CHO).

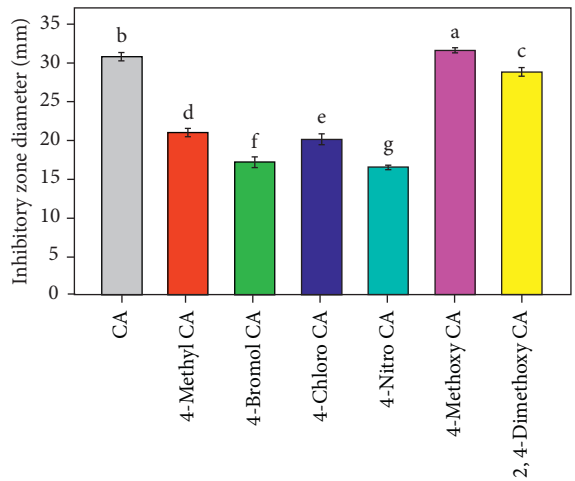


Figure 2: The antifungal activity of CA and its derivatives against *P. digitatum* (each compound concentration was 8 mg/mL).

fashion (Table 3). Increasing the concentrations of 4-methoxy CA, the spore germination decreased significantly. When the concentration reached 15.6 μg/mL, the spore germination was inhibited by 27.50%, whereas 250 μg/mL 4-methoxy CA treatment roughly inhibited the spore germination, reached an inhibition by 92.87%. A logarithm model ($y = 2.247 + 1.501x$, $R^2 = 0.92$) was used to describe the relationship between 4-methoxy CA concentrations and SGI after 12 h of incubation. The half effective concentration (EC_{50}) and 95% effective concentration (EC_{95}) values of 4-methoxy CA were calculated to 39 μg/mL and 391 μg/mL, respectively (Table 3). The results indicate that 4-methoxy CA significantly inhibited the spore germination of *P. digitatum in vitro*.

Table 2: Inhibition effects of 4-methoxy CA and CA against *P. digitatum*.

Concentration (mg/mL)	Inhibition zone (mm)	
	4-Methoxy CA	CA
0	0.00 ± 0.00	
2	20.65 ± 0.49a	19.80 ± 0.28b
4	27.75 ± 0.35a	26.55 ± 0.21b
6	29.65 ± 0.49a	28.10 ± 0.82b
8	32.25 ± 0.35a	31.40 ± 0.57b
10	32.85 ± 0.21a	31.75 ± 0.35b

3.4. Optical Microscopy Observation. The morphology of *P. digitatum* individual hyphae exposed to 4-methoxy CA (0, MIC and MFC) was examined using an optical microscope. The microscopic images exposed the morphological structures of control mycelium found normal having smooth surface (Figure 3). Upon treating with 4-methoxy CA at MIC, the *P. digitatum* mycelium was observed having irregular swellings (Figure 3) and emptied mycelial cells when treated with 4-methoxy CA at MFC, clearly indicated that the rupture of cellular membranes lead to leakage of the cytoplasmic inclusions and death of the cell (Figure 3). Obviously, 4-methoxy CA treatment resulted in damage to *P. digitatum* mycelia.

3.5. Effect of 4-Methoxy CA on Membrane Permeability. The extracellular conductivity of *P. digitatum* suspension treated with a range of concentrations (0, 1/2 MIC, MIC, 2 MIC, and MFC) of 4-methoxy CA for multiple time (0–120 min) fashion is shown in Figure 4. Following the different treatment levels, immediately but significant rise in the extracellular conductivity was seen. After about half an

Table 3: Effect of different 4-methoxy CA concentrations on germination of spores of *P. digitatum* after incubation at 27 ± 1°C for 12 h.

Concentrations (μg/mL)	Spore germination	SGI	Toxic regression equation	Effective concentration (EC, μg/mL)
0	90.48 ± 0.46a	0f		
15.6	65.60 ± 0.74b	27.50 ± 0.65e		
31.3	56.15 ± 0.82c	37.94 ± 0.75d	$y = 2.247 + 1.501x$	$EC_{50} = 39$ (22–43)
62.5	30.00 ± 0.69d	66.84 ± 0.47c	$R^2 = 0.92$	$EC_{95} = 391$ (210–1415)
125	19.82 ± 1.10e	78.09 ± 0.42b		
250	6.45 ± 0.35f	92.87 ± 0.02a		

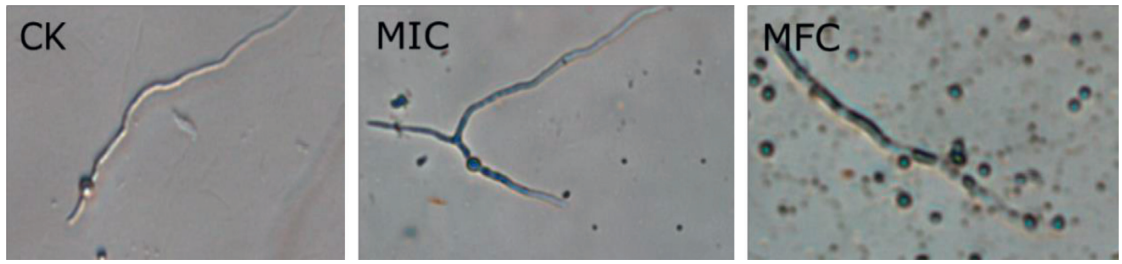


Figure 3: Effect of 4-methoxy CA on hyphal morphology of *P. digitatum*. Control, untreated mycelia; MIC, mycelia treated with 4-methoxy CA at 31.3 μg/mL; MFC, mycelia treated with 4-methoxy CA at 125 μg/mL.

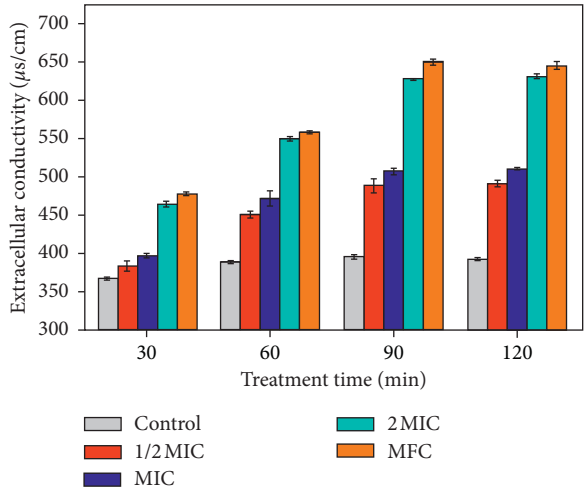


Figure 4: Effect of 4-methoxy CA on extracellular conductivity of *P. digitatum* suspensions. Values are mean ± SE ($n = 3$).

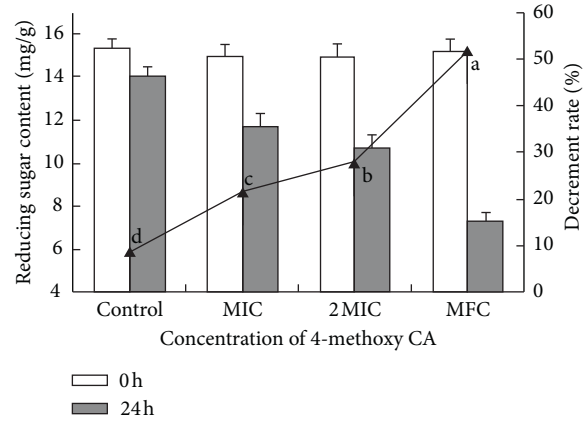


Figure 5: Effect of 4-methoxy CA on reducing sugar content of *P. digitatum* mycelia. Values are mean ($n = 3$) ± SE.

hour of 4-methoxy CA treatment, extracellular conductivity was measured which ranged from 1/2 MIC to MFC, 383.0 to 477.3 μs/cm, a significant ($p < 0.05$) increase than its respective control (361 μs/cm). The increase in the treatment duration helped a marked increase in the extracellular conductivity. Following 2 h of treatment, the extracellular conductivity in *P. digitatum* suspensions exposed to 4-methoxy CA at 1/2 MIC, MIC, 2 MIC, and MFC showed a sharp increase (491.0 ± 4.4, 510.3 ± 1.5, 631.3 ± 3.1, and 645.3 ± 5.0 μs/cm) demonstrating that using high dose of 4-methoxy CA may lead to significant growth by triggering the accelerated cell disruption in *P. digitatum* suspensions.

3.6. Effect of 4-Methoxy CA on Reducing Sugar Content. In order to assess whether the biosynthesis and production of intracellular inclusions have been damaged after 4-methoxy CA treatment, the level of reducing sugars was examined. The levels of reducing sugars of 4-methoxy CA treated *P. digitatum* mycelia at MIC, 2 MIC, and MFC were 11.67 ± 0.70 mg/g, 10.67 ± 0.65 mg/g, and 7.33 ± 0.38 mg/g, respectively, reasonably lower compared with the control group at 24 h of incubation (14.03 ± 0.53 mg/g) (Figure 5), which indicated that the biosynthesis and production of reducing sugar content restrained. It is widely accepted that the decrease of reducing sugar content disturbs energy metabolism. In this case, 4-methoxy CA treatment caused the decrease of reducing sugar, being a direct danger to energy supply.

4. Conclusions

Cinnamaldehyde and its derivatives bear diverse pharmacological activities. In the current study, we synthesized multiple derivatives of cinnamaldehyde *viz.* 4-nitro CA, 4-chloro CA, 4-bromo CA, 4-methyl CA, 4-methoxy CA, and

2,4-dimethoxy CA, but 4-methoxy CA showed highest antifungal actions for citrus fruit postharvest rotting fungi *Penicillium digitatum* (Green mold). Moreover, 4-methoxy CA reduces the spore germination and growth by damaging the fungal cell membrane, as well as declines the levels of reducing sugars. It appears that 4-methoxy CA is a good antifungal agent and can be used for postharvest storage of citrus fruits.

Data Availability

All data used to support the findings of this study are included within the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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Research Article

Oxidative Stability, Color, and Physicochemical and Sensorial Properties of Raw Stacked and Ground Meat Treated with Shahpouri Orange Juice

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Shahpouri orange juice (SOJ) is a rich source of bioactive compounds including flavonoids and phenolic acids. However, limited studies have been done to determine its effect on stacked and ground meat quality. The study was performed to determine and compare the effects of 0, 200, 400, 600, and 800 ppm SOJ with 200 ppm BHA on stacked and ground beef quality. The flavonoid compounds of SOJ were quantified as well as its antioxidant activity. Surface color, pH, lipid oxidation (peroxide value (PV) and thiobarbituric acid (TBA)), and sensorial properties of stacked and ground beef were determined at a day of SOJ incorporation and then after 6 days of storage at 4°C. The addition of SOJ affected pH compared to the control sample. Incorporating SOJ in stacked and ground meat improved redness and decreased lipid oxidation (PV and TBA) during storage compared with control. SOJ at 800 ppm improved overall sensorial properties after 6 days of storage. These results suggested that SOJ could be used as a natural antioxidant in stacked and ground meat to limit lipid oxidation and discoloration.

1. Introduction

Meat and meat products undergo quality loss due to oxymyoglobin (OxyMb) and lipid oxidation, as well as microbial growth. These phenomena cause adverse effects on the nutritional, taste, color, and textural properties of the meat [1, 2]. Toxic compounds can also be produced as a result of oxidation and microbial activity. Grinding meat makes it more prone to chemical oxidation due to the changes in the integrity of the muscle cells membrane and more exposure of fats to oxygen [3], which cause more facilitated interactions of unsaturated fatty acids and prooxidant molecules followed by free radicals production and rancidizing the meat fat [4]. The application of antioxidant and antimicrobial components is a suitable method for improving the quality of meat products.

Synthetic and natural antioxidants can control the lipid oxidation in meat and meat products and improve their quality [1]. The international regulations support the application of a limited amount of synthetic antioxidants to protect meat and meat products from oxidation, due to confirmed severe impact on human health [5]. Therefore, focus on the application of natural antioxidants sourced from fruits, vegetables, herbs, and spices increased. The efforts continue among scientists to find new natural sources of antioxidants that potentially could be added to human food [5–7]. Fruits juice is a good source of antioxidant components for application in meat products.

The people of any age would supply the nutritional needs of their body through consuming the whole fruit or the juice [8]. Based on the report of the United States Department of Agriculture (USDA), orange juice is the most popular produced fruit juice with more than 50% of the international world trade market [9, 10]. The dominant antioxidant compound in orange fruit juice is ascorbic acid or vitamin C followed by flavanone and phenolic acids [11]. Besides the nutritional value and health effects, orange juice phenolic compounds also play a significant role in improving other

food flavor and color [12]. Based on our study there is not any related research on the application of orange juice in stacked and ground meat.

Citrus is one of the most important fruit groups produced in the world which have different characteristics such as the longer maturation period, keeping fruits on the tree after maturity, and the different qualities of the nutritional values. Citrus cultivation is mainly carried out between 40° north latitude and 40° south latitude in the world [13–15]. Although its homeland is reported to be tropical and semitropical regions, production is mainly concentrated in the subtropical climatic zones [16, 17].

Phenolic compounds are secondary plant metabolites that can enter the human diet through the consumption of vegetal products. Importantly, a wide range of health-related activities has been reported for phenolic compounds. In this sense, the phenolic compounds present in citrus fruits have received attention due to their antioxidant, anti-inflammatory, and cardioprotective activities [18–20].

Shahpouri orange is an orange variety harvested in Iran. Due to the high content of flavonoids, Shahpouri orange juice (SOJ) can be considered as a good source of natural antioxidants in the food industry. The main aim of this study was to evaluate the incorporation of SOJ into stacked and ground meat for the first time and investigate its effect on lipid oxidation, color parameters, pH, and sensorial properties.

2. Material and Methods

2.1. Materials. Fresh harvested Shahpouri orange fruits were obtained from a local supermarket in November 2019 (Shiraz, Iran). Acetic acid, chloroform, potassium iodide, sodium thiosulphate, sodium acetate, sodium carbonate, hydrochloric acid, ethanol, methanol, potassium chloride, calcium chloride, dehydrate barium chloride, iron (II) sulfate, ammonium thiocyanate, starch, n-hexane, and plate count agar were purchased from Merck (Darmstadt, Germany). Other chemicals such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Tris buffer, Folin–Ciocalteu reagent, thiobarbituric acid, trichloroacetic acid, and BHA were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Orange Juice Extraction. The Shahpouri orange fruit was stored at 20–25 °C under 70–90% relative humidity until further action. The orange fruits were juiced (Model 4161; Braun, De'Longhi Braun Household GmbH, Hungary) and used for further selected quality tests [21].

2.3. SOJ Phenolic Compounds. The phenolic compounds were quantified using high-performance liquid chromatography (HPLC, PLATIN blue KNAUER, Germany) Güçlü [22]. ODS-2 C18 column (4 × 250 mm), combined with Sphere-image 80–5 precolumn (CA, German), was used for this purpose. A gradient of methanol-water (75 : 25) and acetonitrile/dichloromethane/methanol (70 : 20 : 10) were used as mobile phases A and B, respectively. The setting for mobile phase gradient was 0% B; 5 min, 5% B; 20 min, 60% B; 40 min, 60% B; 50 min, 100% B; 60 min, 100% B; and 70 min, 0% B at 1 ml/min flow rate. Phenolic compounds were identified and quantified through comparison with the standards. The standard calibration curves were built using a minimum of four concentration levels of each standard, with coefficients of correlation ranging from 0.995 to 0.999. The phenolic compounds were reported as mg of component per gram of dried SOJ.

2.4. Antioxidant Properties of SOJ

2.4.1. Reducing Power. Juice (50–500 μg) was mixed with 1 mL of distilled water and added into 5 mL of phosphate buffer (0.2 M, pH 6.6) and 1% aqueous solution of potassium ferricyanide (50 : 50 v/v). The final mixture was incubated at 50°C for 30 min. After the addition of 2.5 mL trichloroacetic acid (10%), the mixture was centrifuged at 3500 rpm for 10 min. Then, 2.5 mL of the supernatant was added into a mixture of distilled water and $FeCl_3$ (0.1%) in a 5 : 1 ratio, respectively. The absorbance of the final solution was measured using a spectrophotometer at 700 nm (Spec 1650PC, Shimadzu, Japan) [23].

2.4.2. Radical Scavenging Activity (RSA). 0.1 mL of SOJ sample was blended with 3.9 mL of a DPPH methanolic solution (25 mg/L). The sample solution was kept in dark for 30 min at 25°C and the absorbance was measured at 517 nm using a spectrophotometer (Spec 1650PC, Shimadzu, Japan) [24]. The percent of DPPH° inhibition (%) was calculated as follows:

$$I\% = \frac{(A_c - A_s)}{A_c} \times 100. \quad (1)$$

A_c and A_s represent blank and sample absorbance, respectively.

2.5. Sample Preparation. Fresh cow meat samples were bought from a local market (Shiraz, Iran) and kept in sealed polystyrene ice-boxes to transport to the laboratory. Then, meat was minced using a meat grinder (Philips, HR2743, Amsterdam, Netherlands). Stacked meat samples were cut in the same size with 1 cm diameter. The SOJ sample (0, 200, 400, 600, and 800 ppm) and BHT (200 ppm) were incorporated into the ground meat while mixing. The stacked meat sample was immersed and covered by SOJ at different concentrations (0, 200, 400, 600, and 800 ppm) and BHT (200 ppm) for 4 min.

2.6. Physicochemical Properties of Meat

2.6.1. pH. 10 g of meat sample was mixed and homogenized with 50 mL deionized water for 1 min. A digital pH meter

(Suntex TS-1, Taiwan) with Ingold electrode was used to determine the pH value of samples at 25°C [25].

2.6.2. PV. The PV values of samples were measured according to the method of Alizadeh-Sani et al. [26]. 0.3 mL of samples was added to 1.5 mL of isooctane/2-propanone solution (3 : 2 (v/v)) and mixed for 30 s. The mixture was then centrifuged at 2000 g for 2 min. 200 μL of clear upper phase was blended with 2.8 mL of methanol: 1-butanol (2 : 1 (v/v)) and 30 μL of thiocyanate/Fe^{2+}. The samples were kept for 20 min at 25°C and the absorbance was read at 510 nm (Spec 1650PC, Shimadzu, Japan). PV was quantified according to an obtained standard curve from cumene hydroperoxide. Peroxide value was reported based on meq of O_2 per kg of lipid.

2.6.3. TBA. Secondary lipid oxidation products were monitored by measuring thiobarbituric acid reactive substances (TBARS) values as the method described by Turgut et al. [27]. 4 g of meat sample was mixed with 20 mL TCA solution (20% w/v) and then centrifuged at 3000 g for 10 min. Afterward, 2 mL of upper phase was separated, mixed with 2 mL TBA solution (0.1% w/v in double-distilled water), and then heated in a boiling water bath at 100°C for 30 min. After cooling to room temperature, the absorbance was read at 520 nm using a spectrophotometer (Spec 1650PC, Shimadzu, Japan). The TBARS values were calculated as mg of malonaldehyde per kg of the sample according to the standard curve of 1, 1, 3, 3-tetraethoxypropane.

2.6.4. Color Properties. The color of the sample was determined using the method described by Hosseini et al. [28]. Photos were taken using a digital camera (Canon PowerShot A540, 6 megapixels resolution) at certain conditions under natural daylight source (6500 K) and then saved as JPG format. The values of color parameters including L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) of each meat sample were determined using Adobe Photoshop® CS6.

2.6.5. Sensorial Properties. Organoleptic characteristics were studied by 20 male and female trained panelists aged between 23 and 28 years, from the Department of Food Science and Technology (Shiraz University). Panelists were selected according to their previous experiences in evaluating detailed differences in the sensorial properties of meat and meat products. In this study, meat samples were cooked at 150°C using a forced draft oven to reach 72°C in core temperature and then maintained warm in the oven for 5 min before sensorial measurements. Rectangular pieces (2×2 cm^2) of samples were cut and then presented at 25°C. Each panelist randomly tested three pieces of all samples and asked to describe their opinions by assigning a liking score between 1 and 9 for the following attributes: taste, 1 (imperceptible) to 9 (extremely intense); texture attributes and juiciness 1 (extremely dry) to 9 (extremely moist); appearance, 1 (extremely soft) to 9 (extremely tough); odor 1 (imperceptible) to 9 (extremely intense), and overall score from 1 (dislike very much) to 9 (like very much). Tap water was served to panelists to rinse their mouths between different formulations [29].

2.7. Statistical Analysis. The average values were analyzed using a one-way analysis of variance (ANOVA) at a significance level of 5%. The significant differences among average values were investigated through Duncan's multiple range tests using SAS software (ver. 9.1, SAS Institute Inc., Cary, NC, USA.).

3. Results and Discussion

3.1. Orange Juice Properties

3.1.1. Phenolic Compounds. The dominant quantified flavonoids and phenolic acids were hesperidin, narirutin, chlorogenic acid, naringin, caffeic acid, and *p*-coumaric acid with 152.20, 35.23, 25.02, 11.99, 11.88, and 5.54 mg/g dried SOJ, respectively (Table 1). The obtained results were in accordance with the results reported by Vanamala et al. [30] and Fusco et al. [31] and confirmed; hesperidin and narirutin are the main flavonoid compounds in orange juice obtained from various orange cultivar.

3.1.2. Radical Scavenging Activity and Reducing the Power of SOJ. The results of radical scavenging activity (%) of SOJ are reported in Table 2. The results showed that, with an increase in the concentration of SOJ from 985 to 3940 ppm, radical scavenging activity significantly increased from 24 to 51%. The reducing power of Shahpouri orange juice in comparison with BHA is reported in Table 3. The results showed that BHA had higher reducing power than SOJ and the 200 ppm BHA and 400 ppm SOJ showed similar reducing power. The significant level of antioxidant activity in orange juice is related to its bioactive compounds such as carotenoids, vitamin C, phenolic acids, and flavonoids [32, 33].

3.2. Meat Sample Properties

3.2.1. pH. The pH of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 4. The results showed that the pH of stacked and ground meat samples was increased in control samples during storage. An increase in pH is associated with the accumulation of ammonia caused by the degradation of proteins and amino acids [34]. THE addition SOJ and BHA had significant effects on keeping the pH of meat samples at a lower level compared with control sample. However, the results indicated that there were no significant differences between SOJ and BHA. Similar results were reported on the effects of different antioxidants on the meat pH by Amiri et al. [35] and Hashemi Gahruie et al. [29].

Table 1: Phenolic compounds of Shahpouri orange juice (SOJ).

Compounds	Concentration (mg/g dried SOJ)
Chlorogenic acid	25.02 ± 0.97C
Caffeic acid	11.88 ± 0.72D
p-Coumaric acid	5.54 ± 0.63E
Narirutin	35.23 ± 0.37B
Naringin	11.99 ± 0.55D
Hesperidin	152.20 ± 1.85A

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column indicate significant differences ($p < 0.05$).

Table 2: Radical scavenging activity (%) of Shahpouri orange juice (SOJ).

Concentration (ppm)	RSA (%)
985	24.00 ± 1.24 C
1970	39.66 ± 0.72B
3940	51.00 ± 1.25 A

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column indicate significant differences ($p < 0.05$).

Table 3: Reducing power of Shahpouri orange juice (SOJ).

Concentration (ppm)	BHA	SOJ
200	0.211 ± 0.002Da	0.101 ± 0.002Db
400	0.393 ± 0.002Ca	0.201 ± 0.002Cb
600	0.636 ± 0.001Ba	0.334 ± 0.002Bb
800	0.847 ± 0.001Aa	0.461 ± 0.001Ab

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences ($p < 0.05$).

3.2.2. Peroxide Value. Peroxide value of stacked and ground meat samples at a day of SOJ incorporation and then after 6 days of storage at 4°C are reported in Table 5. The results showed that the peroxide value of stacked and ground meat samples was increased in control samples after 6 days of storage. The addition of SOJ and BHA had significant effects on the peroxide value of samples in comparison with control. The results showed that a sample containing 400 ppm of SOJ had a similar antioxidant activity with a sample containing 200 ppm BHA. Also ground meat samples showed higher lipid oxidation in comparison with stacked meat.

The oxidation process can be prevented by phenolic compounds and anthocyanin by blocking the processing of free fatty acids formation and free radicals interaction [36]. Therefore, plant extracts rich in phenolic compounds and anthocyanin compounds are usually used in meat products for this purpose. Some researchers reported successfully using plant extract rich in natural antioxidant in meat products during chilled storage including Mediterranean berries, blackcurrant extract, and artichoke extract in pork burger patties, raw pork patties, and in raw beef patties, respectively [37–40], and apple peel-based edible coating, pomegranate peel extract, and pistachio green hull extract in beef [25,41]. A 75% reduction in the oxidation of ground goat meat by using pomegranate rind powder containing 300 ppm phenolic compounds was also reported by Devatkal and Narsaiah [42].

3.2.3. Secondary Oxidation (TBA). One of the adverse effects of lipid oxidation is changing the meat and meat products flavor, which limits its acceptance [43]. Greene and Cumuze [44] revealed that 0.2–0.6 mg MDA/kg of TBARS caused the oxidized flavor in beef and decreased acceptability of the meat tested by panelist. The threshold of 2 mg MDA/kg beef was reported to be acceptable by Campo et al. [45].

TBA value of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 6. The results showed that the TBA values were increased in all samples especially in control samples. This increase was higher in ground meat samples compared to stacked meat. In this study meat rancidity started at the values of 0.4–0.6 mg of malonaldehyde/kg, which was similar to the result of the previous study on pork meat refrigerated for 7 days [46, 47]. The addition of SOJ and BHA had significant effects on the TBA value of samples in comparison with control. It is indicating that the SOJ protected the samples from lipid oxidation. The results showed that a sample containing 600 ppm of SOJ had a similar antioxidant activity with a sample containing 200 ppm BHA. The antioxidant effect of SOJ can be attributed to its bioactive compounds such as flavonoids (mainly hesperidin), phenolic acids, and ascorbic acid [48, 49]. Similar results were reported by Villalobos-Delgado et al. [46] and Firuzi et al. [50] on lipid oxidation of treated meat with different types of vegetable extract.

3.2.4. Color Properties. Color properties ($L^*, a^*, and\ b^*$) of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 7. The results showed that storage time and antioxidant level had no effect on the L^* value of both meat samples. In meat products, higher fat content generally causes more light reflection and consequently brighter color and higher L^* value [51]. The a^* value, indicating redness of samples, decreased for the control sample during the storage time, whereas samples containing either BHA or SOJ did not show any changes. This finding indicated that adding SOJ containing antioxidants could keep the preferred meat red color stable and prevent discoloration.

The red color in meat products is related to OxyMb concentration [52]. Conversion of OxyMb to metmyoglobin (MetMb) causes changes in color from bright red to brown and, consequently, decreases in a^* value. This change in color depends on lipid oxidation and the addition of antioxidants can retard this discoloration process by delaying OxyMb deterioration and slowing down the formation of MetMb [53]. In accordance with our results, it was demonstrated that the

Table 4: pH of stacked and ground meat samples incorporated with Shahpouri Orange Juice (SOJ) and BHA during storage at 4°C for 6 days.

Sample	Stack		Ground meat	
	0	6	0	6
Control	5.50 ± 0.12Ab	5.83 ± 0.07Aa	5.50 ± 0.22Ab	5.83 ± 0.14Aa
BHA (200 ppm)	5.50 ± 0.12Aa	5.50 ± 0.12Ba	5.57 ± 0.07Aa	5.53 ± 0.14Ba
SOJ (200 ppm)	5.53 ± 0.07Aa	5.66 ± 0.07Ba	5.53 ± 0.07Aa	5.63 ± 0.07Ba
SOJ (400 ppm)	5.53 ± 0.07Aa	5.57 ± 0.07Ba	5.53 ± 0.14Aa	5.57 ± 0.07Ba
SOJ (600 ppm)	5.47 ± 0.19Aa	5.50 ± 0.07Ba	5.57 ± 0.07Aa	5.57 ± 0.07Ba
SOJ (800 ppm)	5.53 ± 0.07Aa	5.53 ± 0.09Ba	5.43 ± 0.14Aa	5.42 ± 0.07Ca

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences ($p < 0.05$).

Table 5: Peroxide value (meq/kg of lipid) stacked and ground meat samples incorporated with Shahpouri Orange Juice (SOJ) and BHA during storage at 4°C for 6 days.

Sample	Stacked		Ground	
	0	6	0	6
Control	0.63 ± 0.14Ab	1.83 ± 0.08Aa	0.70 ± 0.06Ab	2.13 ± 0.14Aa
BHA (200 ppm)	0.74 ± 0.11Ab	0.96 ± 0.14Ca	0.73 ± 0.08Ab	1.26 ± 0.21Ca
SOJ (200 ppm)	0.70 ± 0.06Ab	1.25 ± 0.12Ba	0.75 ± 0.17Ab	1.74 ± 0.22Ba
SOJ (400 ppm)	0.71 ± 0.08Ab	1.01 ± 0.14Ca	0.77 ± 0.11Ab	1.30 ± 0.07Ca
SOJ (600 ppm)	0.65 ± 0.11Ab	0.91 ± 0.07Ca	0.69 ± 0.07Ab	1.02 ± 0.08Da
SOJ (800 ppm)	0.60 ± 0.12Aa	0.83 ± 0.12Ca	0.72 ± 0.12Ab	0.94 ± 0.07Da

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences ($p < 0.05$).

Table 6: TBA (mg MDA/kg) stacked and ground meat samples incorporated with Shahpouri Orange Juice (SOJ) and BHA during storage at 4°C for 6 days.

Sample	Stacked		Ground	
	0	6	0	6
Control	0.05 ± 0.00Ab	1.18 ± 0.01Aa	0.06 ± 0.00Ab	1.32 ± 0.02Aa
BHA (200 ppm)	0.05 ± 0.01Ab	0.78 ± 0.03Da	0.06 ± 0.02Ab	0.89 ± 0.02Ca
SOJ (200 ppm)	0.05 ± 0.00Ab	0.93 ± 0.01Ba	0.06 ± 0.00Ab	1.07 ± 0.01Ba
SOJ (400 ppm)	0.05 ± 0.01Ab	0.82 ± 0.03Ca	0.06 ± 0.01Ab	0.96 ± 0.05Ca
SOJ (600 ppm)	0.05 ± 0.00Ab	0.71 ± 0.02Ea	0.06 ± 0.01Ab	0.84 ± 0.03Da
SOJ (800 ppm)	0.05 ± 0.02Ab	0.59 ± 0.02Fa	0.06 ± 0.00Ab	0.72 ± 0.01Ea

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences ($p < 0.05$).

incorporation of 10 mg gallic acid equivalent in pomegranate rind could control color changes in frankfurter during storage at refrigerator [54] which is probably linked with lipid oxidation [55]. There are some reports demonstrating that natural antioxidants may retard color loss by delaying red color deterioration by slowing metmyoglobin formation [56–58].

The obtained b^* value of stacked and ground meat samples was decreased in control samples. The addition of SOJ and BHA had significant effects on the TBA value of samples in comparison with control. The results showed that a sample containing 400 ppm of SOJ had a similar antioxidant activity with a sample containing 200 ppm BHA. Biswas et al. [59] reported that the addition of mint (Mentha spicata) and curry extracts (*Murraya koenigii* L.) in raw ground pork meat stabilized the red color during 12 days of chilled storage. Besides, Turgut et al. [25] showed that the presence of pomegranate extracts rich in phenolic compounds and anthocyanin in pork patties and meat controlled the color which changed efficiently during chilled storage. Firuzi et al. [50] noted that 10 mg gallic acid equivalent in pomegranate rind limited frankfurter color change during chilled storage. However, some reports showed an increase in a^* value during storage at the refrigerator.

3.2.5. Sensorial Properties. The appearance and color are the most important properties that influence the consumers' judgment and acceptance, to purchase meat [46]. Overall sensorial properties of stacked and ground meat samples at a day of SOJ incorporation and after 6 days of storage at 4°C are reported in Table 8. Based on the obtained result, the sensorial properties of the sample containing either BHA or SOJ remained stable during the storage time which was due

Table 7: Color properties of stacked and ground meat samples incorporated with Shahpouri Orange Juice (SOJ) and BHA during storage at 4°C for 6 days.

L^*	Stacked		Ground	
Sample	0	6	0	6
Control	47.31 ± 0.21Aa	48.98 ± 0.65Aa	41.49 ± 1.05Aa	41.86 ± 0.57Aa
BHA (200 ppm)	48.62 ± 0.09Aa	48.09 ± 0.46Aa	44.00 ± 0.41Aa	43.87 ± 0.49Aa
SOJ (200 ppm)	46.02 ± 0.41Aa	47.40 ± 0.89Aa	42.46 ± 0.72Aa	43.55 ± 0.38Aa
SOJ (400 ppm)	47.68 ± 0.74Aa	47.51 ± 0.65Aa	41.68 ± 0.79Aa	42.26 ± 0.86Aa
SOJ (600 ppm)	46.08 ± 0.91Aa	46.22 ± 1.61Aa	42.37 ± 0.88Aa	41.10 ± 1.45Aa
SOJ (800 ppm)	46.50 ± 0.57Aa	47.30 ± 0.67Aa	44.04 ± 0.80Aa	44.97 ± 1.00Aa
a^*	Stacked		Ground	
Sample	0	6	0	6
Control	11.50 ± 0.65Aa	7.27 ± 0.06Bb	12.13 ± 0.44Aa	8.42 ± 0.24Bb
BHA (200 ppm)	12.85 ± 0.53Aa	10.66 ± 0.59Aa	12.17 ± 0.52Aa	10.81 ± 0.95Aa
SOJ (200 ppm)	11.36 ± 0.23Aa	9.61 ± 0.42ABa	13.44 ± 0.53Aa	10.35 ± 0.59Aa
SOJ (400 ppm)	12.48 ± 0.25Aa	10.87 ± 0.65Aa	12.66 ± 0.30Aa	11.48 ± 1.14Aa
SOJ (600 ppm)	12.10 ± 0.77Aa	11.06 ± 0.17Aa	11.30 ± 0.86Aa	11.43 ± 1.17Aa
SOJ (800 ppm)	12.15 ± 0.41Aa	12.19 ± 0.73Aa	13.15 ± 0.36Aa	12.68 ± 0.57Aa
b^*	Stacked		Ground	
Sample	0	4	0	4
Control	6.15 ± 0.44Aa	4.76 ± 0.31Cb	6.54 ± 0.07Aa	4.32 ± 0.12Bb
BHA (200 ppm)	5.77 ± 0.94Aa	5.25 ± 0.22Aa	5.78 ± 0.17Aa	5.84 ± 0.11ABa
SOJ (200 ppm)	5.83 ± 0.53Aa	4.83 ± 0.22Bb	5.66 ± 0.00Aa	4.69 ± 0.66Ba
SOJ (400 ppm)	6.29 ± 0.32Aa	5.46 ± 0.38Aa	7.99 ± 0.73Aa	7.56 ± 0.16Aa
SOJ (600 ppm)	6.86 ± 0.64Aa	6.78 ± 0.30Aa	6.91 ± 0.77Aa	6.85 ± 0.83Aa
SOJ (800 ppm)	7.43 ± 0.19Aa	7.03 ± 0.12Aa	6.55 ± 0.33Aa	5.94 ± 0.28Aa

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences ($p < 0.05$).

Table 8: Overall sensorial properties of stacked and ground meat samples incorporated with Shahpouri Orange Juice (SOJ) and BHA during storage at 4°C for 6 days.

Sample	Stacked		Ground	
	0	6	0	6
Control	7.33 ± 0.71Aa	5.33 ± 0.71Bb	7.00 ± 1.24Aa	5.33 ± 0.71Bb
BHA (200 ppm)	7.67 ± 0.33Aa	7.00 ± 0.33Aa	7.33 ± 0.72Aa	7.00 ± 0.33Aa
SOJ (200 ppm)	7.00 ± 1.24Aa	6.33 ± 1.24Aa	6.33 ± 0.24Aa	5.00 ± 1.24Aa
SOJ (400 ppm)	7.67 ± 1.43Aa	7.33 ± 1.43Aa	7.00 ± 1.24Aa	7.67 ± 1.43Aa
SOJ (600 ppm)	8.00 ± 1.24Aa	7.00 ± 1.24Aa	8.33 ± 0.72Aa	7.67 ± 1.24Aa
SOJ (800 ppm)	7.33 ± 1.43Aa	7.67 ± 1.43Aa	8.33 ± 1.43Aa	7.33 ± 1.43Aa

*Data represent mean ± standard deviation of three independent repeats. **Different capital letters in each column and lowercase ones in each row indicate significant differences ($p < 0.05$).

to the prevention of MetMb formation. Decreasing OxyMb and increasing MetMb in meat induces a sense of staleness and decrease acceptability. In contrast, the control sample showed a significant decrease in sensorial properties [60].

4. Conclusion

Incorporation of SOJ in stacked and ground meat decreased lipid oxidation due to the presence of antioxidant compounds in SOJ and prevent decreasing redness during the 6 days of storage at 4°C. It was also revealed that the addition of SOJ at different levels could improve the sensorial properties of samples compared to that of control. Considering these results, SOJ has the potential to be used as a natural additive in meat products to improve their quality during chilled storage.

Data Availability

All data and analyses are reported in the table and figure.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Prediction and Identification of Antioxidant Peptides in Potato Protein Hydrolysate

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Principal component analysis (PCA) was used to cluster the possible amino acid compositions of antioxidant peptides in potato protein hydrolysate (PPH). The antioxidant peptides exhibiting high $ABTS^{+\bullet}$ scavenging capacity were isolated with the procedure of ultrafiltration, gel filtration, and preparative RP-HPLC and identified by UPLC-MS/MS. Phe, Tyr, and His were shown to group together with $ABTS^{+\bullet}$ scavenging capacity in component matrix plot. Three prominent peptides, namely, Phe-Tyr, Tyr-Phe-Glu, and Pro-Pro-His-Tyr-Phe, which matched the sequence of patatin and were made up of Phe and Tyr, were identified. The peptide Tyr-Phe-Glu demonstrated antioxidant activity against Caco-2 cell oxidation induced by H_2O_2. The results suggested that multivariate analysis could be used to predict the amino acid compositions of antioxidant peptides.

1. Introduction

Enzymatic hydrolysis has been used to improve the solubility of commercial potato protein with low solubility [1, 2]. The radical scavenging and metal chelating capacity [2] of potato protein hydrolysate (PPH) have resulted in its potential role in retarding lipid oxidation of food products. It was demonstrated that PPH was able to suppress the lipid oxidation in cooked beef patties [2], cooked frankfurters [3], and food model emulsions [4]. As protein hydrolysates are a mixture of peptides, exploring the exact structure of antioxidant peptides in the potato protein hydrolysate is of interest. That will help to explain their antioxidant mechanisms in food products and contribute to the relationship between structure and activities of peptides.

Waglay and Karboune have identified a lot of peptides from PPH prepared using Flavourzyme and papain [5]. Those peptides matched the sequence in potato protein. However, their bioactivities were not estimated. Besides, few peptide sequences were related to antioxidant peptides. Several antioxidant peptides have been identified in previous research [6–8], while little has been done to predict the amino acid compositions of antioxidant peptides before identification. Bioinformatics methods might be a promising method for predicting the potential sequence of bioactive peptides in potato protein [9–12]. Unfortunately, release of these bioactive peptides from potato protein using proteases was not achieved.

Since antioxidant activity of peptides is related to the composition and sequence of the amino acids, classifying amino acid composition may be able to predict the sequence of the antioxidant peptides. Multivariate analyses such as principal component analysis (PCA) have been used to differentiate and cluster the samples in food science and technology [13–17] and predict the bioactive peptides presented in food proteins [18]. In silico analysis based on the data from databases and literature was used in these studies. Little has been done to use the data collected from experiments.

In our previous research, we have incorporated PPH fractions with different amino acid compositions into emulsions to retard their lipid oxidation [6, 7]. We hypothesized that the chemical action of PPH may be related to antioxidant peptides in these fractions. Furthermore, some special amino acids may make up or contribute a lot to these antioxidant peptides. Therefore, the aim of the present

research was to predict possible amino acid compositions in antioxidant peptides presented in PPH using PCA through amino acid compositions of those fractions. Then, the individual antioxidant peptides were identified to verify our assumption. The antioxidant capacity of synthesized antioxidant peptide was evaluated by H_2O_2 induced cell oxidation model.

2. Materials and Methods

2.1. Materials. Potato protein concentrate containing 81% protein was obtained from AVEBE (Veendam, Netherlands) and used without further purification. Alcalase (endoproteinase from *Bacillus licheniformis*, 2.4 AU/g), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), and Cell Counting Kit-8 were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The synthesized peptide YFE (Tyr-Phe-Glu) with the purity of 98% was obtained from Top-peptide Bio Co., Ltd. (Pudong, Shanghai, China). Caco-2 human intestinal cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Gibco Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), nonessential amino acids (NEAA), penicillin, and streptomycin were purchased from Thermo Fisher Scientific (China) Inc. (Shanghai, China). Malondialdehyde (MDA) assay kit, protein carbonyl assay kit, catalase (CAT) assay kit, glutathione peroxidase (GSH-PX) assay kit, and superoxide dismutase (SOD) assay kit were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All of the other analytical-grade chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Distilled and deionized water was used to prepare all the solutions.

2.2. Preparation of PPH. PPH was prepared according to the method of Wang and Xiong [2], and the method description partly reproduces their wording. PPH was prepared by hydrolyzing potato protein suspension (40 mg/mL) at pH 8.0 for 60 min at 50°C using Alcalase with enzyme/substrate ratio of 1/100 (w/w). After hydrolysis, the pH of hydrolysate was adjusted to 7.0 with 1 M NaOH, and then the enzyme was inactivated at 80°C for 15 min. The hydrolysate was freeze-dried and stored at 4°C in sealed plastic bags before use.

2.3. Peptide Fractionation with Different Polarity and Molecular Weight. PPH fractions with different polarity were isolated using ammonium sulfate precipitation as in the method described by Cheng et al. [7]. The fractions precipitated with the ammonium sulfate concentration of 30, 50, 70, and 90% were defined as P30, P50, P70, and P90. In addition, PR was the retentate of PPH after ammonium sulfate precipitation at the concentration of 90%. The fractions of peak 1, peak 2, and peak 3 with different molecular weight were separated from PPH using gel filtration as in the method described by Cheng et al. [6]. All the fractions were then freeze-dried and stored at 4°C in sealed bags before use. The amino acid compositions of these fractions were estimated using OPA derivation method [19].

2.4. Peptide Fractionation Using Ultrafiltration. The PPH solution was passed through Millipore Pellicon-2 ultrafiltration system (Millipore Corporation, Billerica, MA, USA) in the order of 10 and 1 kDa molecular weight cut-off (MWCO) ultrafiltration membranes. The retentate from 10 kDa MWCO ultrafiltration membrane (fraction > 10 kDa) and 1 kDa MWCO ultrafiltration membrane (fraction in the range of 10–1 kDa) and the permeate from 1 kDa MWCO ultrafiltration membrane (fraction < 1 kDa) were collected and freeze-dried for further use.

2.5. Isolation and Identification of Active Peptides. The method described by Cheng et al. [6] was followed, and the method description partly reproduces their wording. PPH fraction from 1 kDa MWCO ultrafiltration membrane permeates was isolated using gel filtration (Sephadex G-15), and three fractions were collected. Then the PPH fraction that displayed the highest $ABTS^{+\bullet}$ scavenging activity within those three fractions was further purified on a SunFire Prep C18 column (1.9 × 15 cm) with preparative HPLC (Waters Corporation, Milford, MA). The fractions were eluted using methanol as solvent in linear gradient mode (10–95% in 50 min) at the flow rate of 6 mL/min. The elution fractions were collected at the accuracy of 0.01 min. The procedure was run ten times to get sufficient amounts of peptide fractions. The elution fractions were freeze-dried after removing the methanol.

The purified peptide fractions that displayed the highest $ABTS^{+\bullet}$ scavenging activity were then subjected to ultra-performance liquid chromatography (Waters Corporation, Milford, MA), followed by Waters Synapt Mass Quadrupole Time-of-Flight Mass Spectrometer (Waters Corporation, Milford, MA) for sequence identification. Peptide sequencing program in the MassLynx software was used to interpret the spectra. Potato protein sequences published in National Center for Biotechnology Information (NCBI) database were used to match the peptide sequences.

2.6. Radical Scavenging Activity (RSA). The ABTS radical cation ($ABTS^{+\bullet}$) assay was estimated by the method described by Cheng et al. [6]. In addition, the RSA of peptide samples was expressed as Trolox equivalent (μM).

2.7. Fe^{2+} Chelating Activity. Ferrous metal ion chelating activity was determined according to the method of Wu et al. [20], and the method description partly reproduces their wording. One milliliter of $FeCl_2$ (20 μM) was mixed with 0.5 mL of PPH fraction, and then 1 mL of ferrozine (500 μM) was added. The absorbance of the mixture was recorded at 562 nm (A_s) after 10 min incubation. Deionized water was used as the control to determine the absorbance of the mixture (A_0). The Fe^{2+} chelating activity was calculated as $[(A_0 - A_s)/A_0] \times 100\%$.

2.8. Cell Culture. Caco-2 cells (1×10^5 cells/mL) were cultured in high glucose DMEM with 10% FBS, 1% NEAA, and 50 units/mL of penicillin-streptomycin and incubated in 5% CO_2 at 37°C. Cell passage numbers 20–50 were used in the experiments. The cells were grown in a flask for 6 days with fresh medium replaced every other day.

2.9. Induction of Oxidative Stress. The oxidative stress was induced to confluent cells by adding 0.75 mM H_2O_2. The Caco-2 cell was inoculated into 96-well culture plates at a concentration of 4×10^3–6×10^3 per well and cultured for 24 h. The cells were then incubated for 4 h with various concentrations of YFE (0.25–5 mg/mL). Cells were washed twice with PBS, followed by the addition of 0.75 mM H_2O_2 for 4 h. At the end of 8 h, the cell viability was determined using Cell Counting Kit-8. Each plate tested included negative control (NEG, treated with treatment medium only) and positive control (POS, treated with treatment medium and H_2O_2).

2.10. Determination of Lipid Peroxidation and Protein Carbonyl Groups of Caco-2 Cell. The cellular MDA and the protein carbonyl groups were measured to determine the extent of lipid peroxidation and protein oxidation under oxidative stress induced by H_2O_2, respectively. MDA and protein carbonyl were determined using kit according to the manufacturer's instructions.

2.11. Activity Determination of Antioxidant Enzyme. CAT, GSH-PX, and SOD activities were determined using kit according to the manufacturer's instructions.

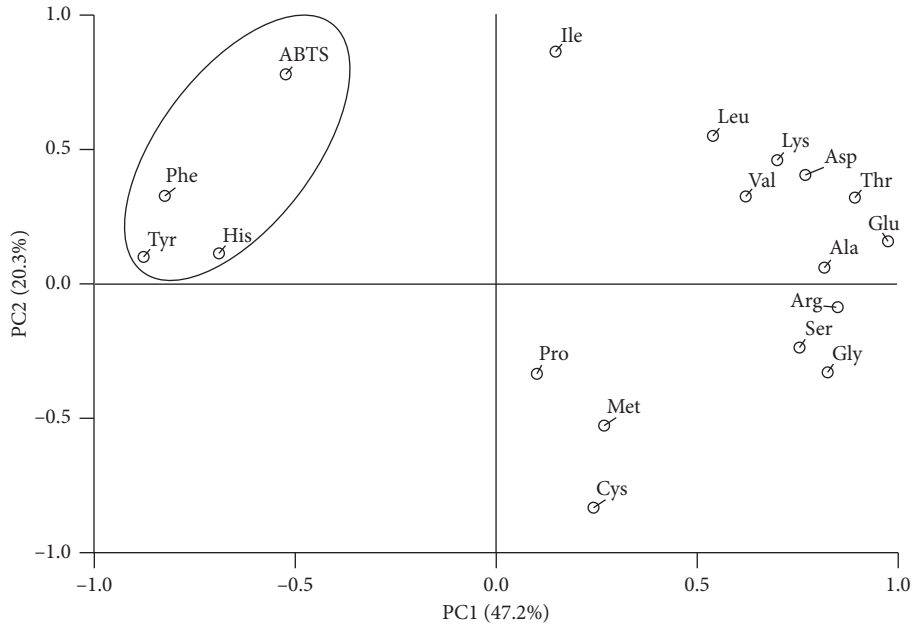
2.12. Statistical Analysis. All assays were carried out in at least three different trials, and data were subjected to analysis of variance (ANOVA). When treatment effects were found significant ($p < 0.05$), the difference between means was identified by Tukey's test. Amino acid compositions and ABTS radial scavenging capacity of PPH fractions with different molecular weight and polarity were used for principal component analysis (PCA). PCA was performed by SPSS 17.0 (SPSS Inc., Chicago, IL).

3. Results and Discussion

3.1. Principal Component Analysis. In our previous research, three fractions (peak 1, peak 2, and peak 3) were separated from PPH using Sephadex G-15 gel filtration [6], and five fractions were separated from PPH using ammonium sulfate precipitation [7]. These fractions were shown to retard lipid oxidation of emulsions. That might be related to their free radical scavenging capacity. Although some antioxidant peptides were identified from these fractions, whether it was possible to predict the sequence of antioxidant peptides by amino acid composition was not clear. In many studies, amino acid composition has been used to assess antioxidant capacity of protein hydrolysates. However, little has been done to use amino acid compositions to predict sequence of antioxidant peptides. To distinguish the effective antioxidant amino acids and predict the antioxidant peptide compositions in PPH, the association of amino acid compositions in those fractions (Table S1) was assessed using principal component analysis (PCA) method. As a result, four components with a cumulative variance of 93.0% were extracted by PCA (Figure S1). The variance of PC1, PC2, PC3, and PC4 was 47.2%, 20.3%, 16.6%, and 8.9%, respectively. The first two principal components, which were able to explain about 70% of data variance, were used to analyze potential amino acid components of antioxidant peptides in PPH. The component matrix plot of PC1 and PC2 is shown in Figure 1(a). The result showed that the amino acids in different PPH fractions were able to separate into several groups. Within these groups, three amino acids, namely, Phe, Tyr, and His, were found to be close to ABTS.

Actually, Phe, Tyr, and His have proved to be important components of antioxidant peptides from different protein hydrolysates [21, 22]. A novel peptide, Pro-Phe-His-Pro-Tyr, which was made up of Phe, Tyr, and His was identified from nest protein hydrolysate [23]. It was suggested that some of the antioxidant peptides in PPH might contain at least one of these three amino acids. Fortunately, \we have identified several antioxidant peptides existing in potato protein in our previous works [6, 7], in which Tyr was common amino acid. However, little was known about the existence of antioxidant peptides which were made up of or based on two or three amino acids from Phe, Tyr, and His, such as Tyr-Phe, Phe-Tyr, and His-Tyr-Phe. To make our hypothesis reasonable, we matched the supposed peptide sequences to the sequence of patatin which was the most abundant protein in potato. The result in Figure 1(b) showed that the peptide sequence of YF, FY, YY, FF, FYF, and HYF (bold) was displayed in the amino acid sequence of patatin. To verify the possible existence of peptides, we carried out the common procedure to identify the antioxidant peptides in PPH in the following sections.

3.2. Ultrafiltration and Gel Filtration Fractionation. PPH was first fractionated using ultrafiltration with cut-off molecular weight of 10 and 1 kDa. The $ABTS^{+\cdot}$ scavenging activity and Fe^{2+} chelating activity of fractions at the protein concentration of 1 mg/mL are shown in Figure 2. The fraction with the molecular weight less than 1 kDa indicated the highest $ABTS^{+\cdot}$ scavenging activity and Fe^{2+} chelating activity. Therefore, the fraction with the molecular weight less than 1 kDa was loaded into gel filtration unit. G15 was used to separate the PPH less than 1 kDa, and three fractions (peak 1, peak 2, peak 3) were collected. The fraction peak 3 which had the lowest molecular weight exhibited the highest $ABTS^{+\cdot}$ scavenging activity and Fe^{2+} chelating activity (Figure 3). $ABTS^{+\cdot}$ scavenging activity of peak 3 (1 mg/mL) increased by 92.4%, 95.4%, and 124.8% when compared with PPH (1 mg/mL), BHA (0.1 mg/mL), and Vc (0.05 mg/mL), respectively. Meanwhile, Fe^{2+} chelating activity of fraction peak 3 (1 mg/mL) increased by 6.4 and 3.0 times when compared with PPH (1 mg/mL) and EDTA (1 mg/mL), respectively. The results showed that LMW fractions



(a)

1 mattnsftil ifmilattss tfatlgemvt vlsidgggik giipatilef legqlqevdn
61 ntdarladyf dviggtstgg lltamittpn etnrpfaaak divpfyfehg pkifqssgsi
121 fgpkydgkyl mqvlqeklge trvhqaltev aissfdiktn kpviftksnl akspeldakm
181 ydicystaaa ptffpphyfa tntsngdkye fnlvdgavat vddpallsis vatklaqvdp
241 kfasiksl nykqmlllsl gtgttsefdkty taeetakwgt arwmlviqkm tsaassymtd
301 yylstafqal dsqnnylrvq enaltgttte lddaseanmq llvqvgedll kksvskdnpe
361 tyeealkrfa kllsdrkklr ankasy

(b)

Figure 1: Component matrix plot of amino acid compositions in PPH fractions (a) and amino acid sequence of patatin from NCBI (b).

contributed a lot to the free radial scavenging activity and Fe^{2+} chelating activity of PPH. In this respect, it has been indicated that many antioxidant peptides were small peptides [24–26]. Furthermore, antioxidant peptides identified from PPH were reported to be made up of 2 to 6 amino acids in previous works [6–8].

3.3. Peptide Purification and Sequence Identification. As peak 3 from gel filtration was still a mixture of peptides, it was purified using preparative RP-HPLC. Twenty-three fractions were separated from peak 3 (Figure 4(a)) and labeled from P3-F1 to P3-F23, respectively. According to $ABTS^{+\cdot}$ scavenging activity (Figure 4(b)) and peak area of fractions (Figure 4(b)), three fractions, P3-F6, P3-F8, P3-F14, were selected to identify the peptide sequences by UPLC-MS/MS. Ten peptides whose probability of certainty exceeded 95% were identified. The sequence of these peptides, which were made up of 2 to 5 amino acids with the molecular weight range from 280 to 700, is exhibited in Table 1. The results confirmed that antioxidant peptides in PPH were LMW short peptides; nevertheless, different fractionation procedures were used.

The peptide sequences identified were then matched to the sequence of patatin in the NCBI database. The existence of Leu-Asp-Ala-Lys, Phe-Tyr, Tyr-Tyr, Tyr-Phe-Glu, and Pro-Pro-His-Tyr-Phe was indicated (Table 1).

Besides the size of peptide, amino acid residues were known as an important factor for the antioxidant activity of protein hydrolysates. Tyr and Phe which were prone to oxidation [27] for their aromatic group were the most abundant amino acids in these peptides. Moreover, His might react with proteins [28] to protect proteins from oxidation. When Pearson's correlation was used to analyze the relation between content of individual amino acid and $ABTS^{+\cdot}$ scavenging activity, content of Tyr and Phe also displayed significantly positive relationship with $ABTS^{+\cdot}$ scavenging activity ($p < 0.05$). Furthermore, it was interesting that those peptides except Leu-Asp-Ala-Lys fitted well our hypothesis that antioxidant peptides might be made up of or based on two or three amino acids from Phe, Tyr, and His. Some peptides or fragments including FY, YY, YF, and HYF in patatin were successfully predicted and identified. The MS/MS spectra of the prominent peptides including Phe-Tyr, Tyr-Phe-Glu, and Pro-Pro-His-Tyr-Phe are shown in Figure 5. Phe-Tyr which exhibited the highest peak area in

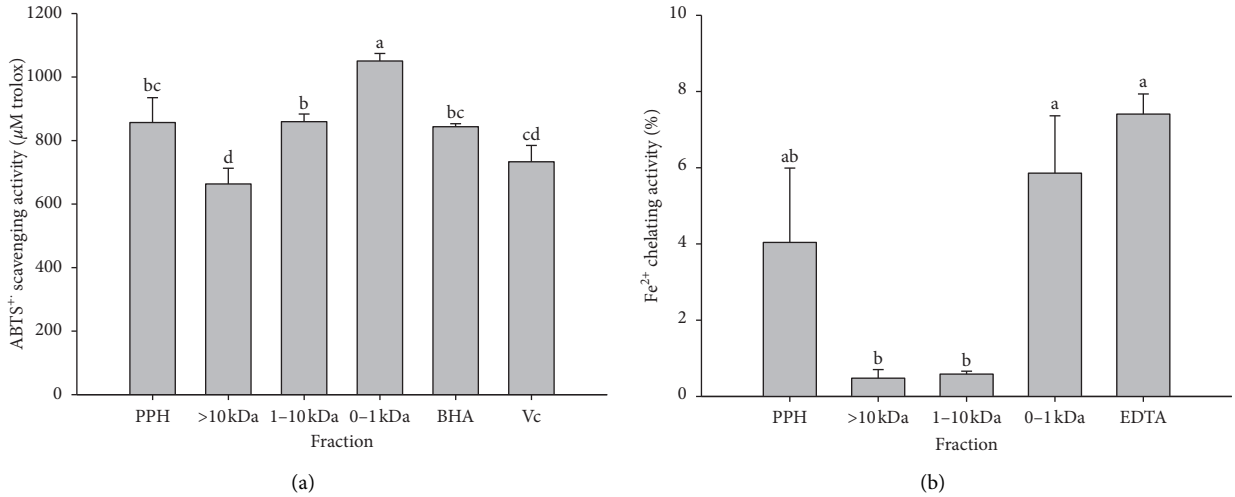


Figure 2: $ABTS^{+\bullet}$ scavenging activity (a) and metal chelating activity (b) of PPH fractions obtained from ultrafiltration using the membrane molecular weight cut-off of 10 and 1 kDa at a protein concentration of 1 mg/mL (the concentrations of BHA, Vc, and EDTA were 0.1, 0.05, and 1 mg/mL, respectively).

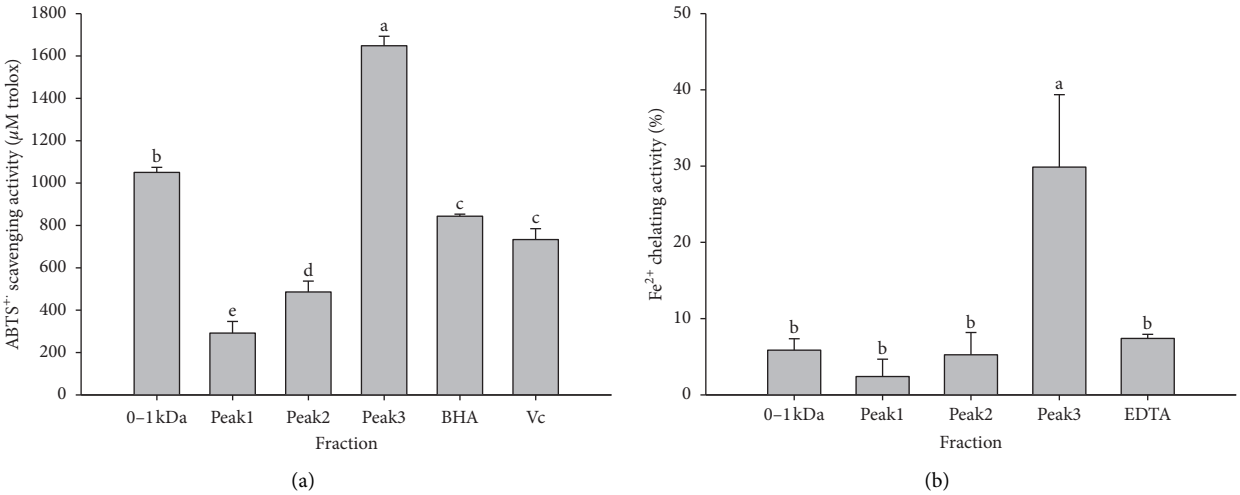


Figure 3: $ABTS^{+\bullet}$ scavenging activity (a) and metal chelating activity (b) of gel filtration fractions from PPH ultrafiltration fractions passing the membrane with the molecular weight cut-off of 1 kDa at the protein concentration of 1 mg/mL (the concentrations of BHA, Vc, and EDTA were 0.1, 0.05, and 1 mg/mL, respectively).

chromatography spectrum was the prominent peptide in fraction P3F8. Interestingly, Phe-Tyr was also found in peptide mixture which was separated from the water phase of emulsion prepared using PPH (not published). It seemed that the peptides we identified at the present work were distributed into water phase of emulsions. In our previous work [29], we have identified some antioxidant peptides partitioned at the interface of emulsion, but none of the peptides reported in the present work was the same as them. Our present result could explain the reason why the antioxidant peptides we identified did not match the interfacial peptides. That confirmed our hypothesis in one of the antioxidant mechanisms of PPH for retarding lipid oxidation of emulsion that PPH partitioning in the water phase was able to prevent free radical transferring through the oil droplets by their free radical scavenging capacity [4]. In a word, our result showed that PCA might be an efficient method to predict the sequence of antioxidant peptides based on amino acid compositions.

Although Ser-Ser-Glu-Phe in P3F14 did not match the sequence of patatin, it was shown to exist in the sequence of metallocarboxypeptidase inhibitor which was one of the protease inhibitors in potato [30]. Moreover, Ser-Ser-Glu-Phe is part of the antioxidant peptide Ser-Ser-Glu-Phe-Thr-Tyr which was identified in the sequence of metallocarboxypeptidase inhibitor [7]. In addition, the remaining part of peptide Thr-Tyr was also identified as antioxidant peptide in PPH [6]. That might be due to the broad cleavage

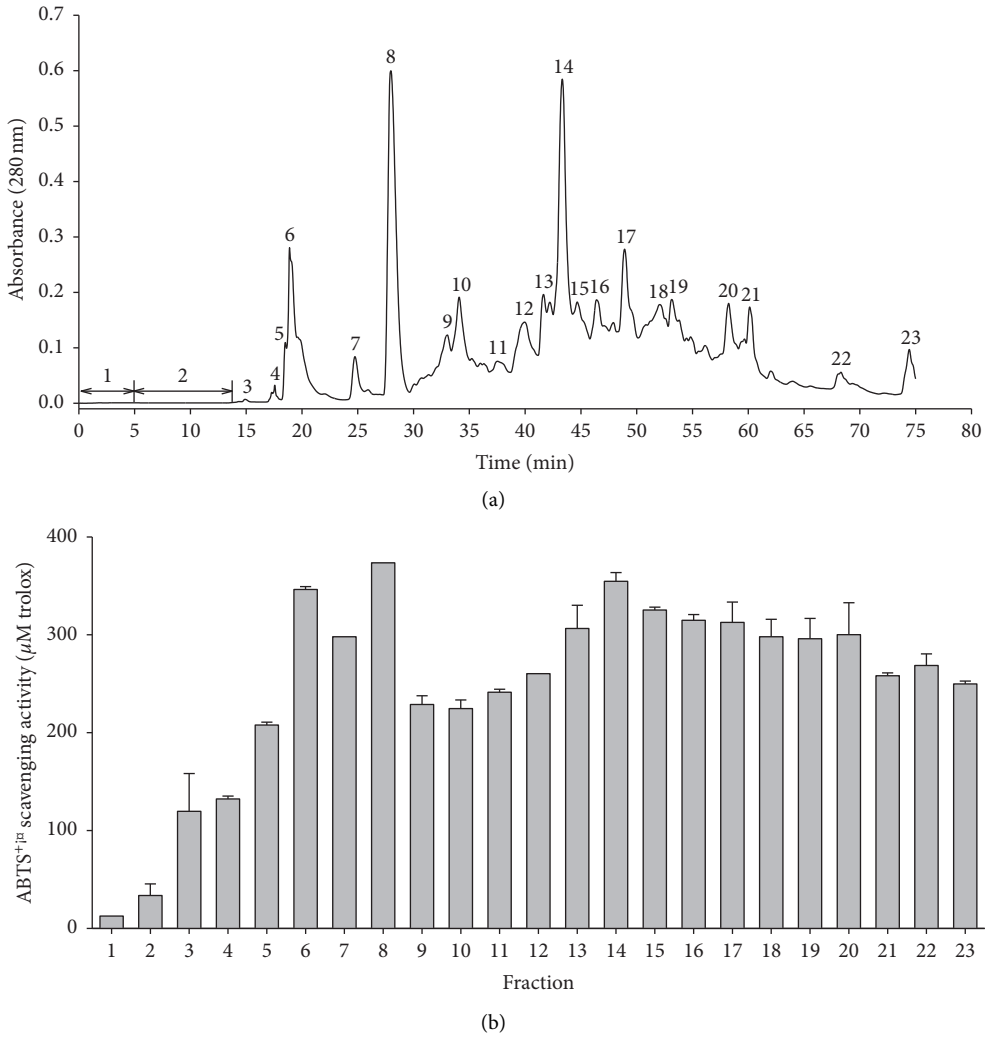


Figure 4: Preparative RP-HPLC spectra of peak 3 obtained from PPH mixture using gel filtration after ultrafiltration with 23 isolated fractions labeled by numbers (a), and ABTS+• scavenging activity of these individual correspondent fractions at the protein concentration of 50 μg/mL (b).

Table 1: Antioxidant peptides from selected fractions in PPH that were isolated and identified using UPLC-MS/MS.

Fraction	Peptide	MW	Prob. (%)	Matched sequence in patatin[a]
P3F6	Ala-Ala-His	297.1	99.99	Not
	Leu-Asp-Ala-Lys	445.3	100.00	f170 akspe**ldak**m
	Ser-Ser-Arg-Tyr	511.2	99.70	Not
P3F8	Phe-Tyr	328.1	100.00	f101 divp**fy**fehg
	Tyr-Tyr	344.1	100.00	f301 **yy**lstafqal
	Gly-Tyr-Pro-Arg	491.2	99.94	Not
P3F14	Ser-Ser-Gly-Phe	396.2	97.99	Not
	Leu-Pro-Thr	329.2	98.80	Not
	Tyr-Phe-Glu	457.2	100.00	f101 divpf**yfe**hg
	Pro-Pro-His-Tyr-Phe	659.3	99.99	f191 ptff**pphyf**a

[a]From National Center for Biotechnology Information (NCBI).

site and priority cleavage at the aromatic amino acids of Alcalase [31]. According to the peptides we identified, Alcalase was indicated to hydrolyze the peptide bonds in potato protein on both amine and carboxyl sides of Tyr and Phe; amine side of Ser, Thr, Leu, and Ile; and carboxyl side of Lys, Glu, Gln, and Met. It was not consisted with the work on casein phosphopeptides released by Alcalase, in which carboxyl side of Glu, Met, Leu, Tyr, Lys, and Gln was the cleavage site [32]. However, the peptides SIDGGGIK (f33-f40), TNKPVIF (f159-f167), and SNLAKSPE (f168-f1175)

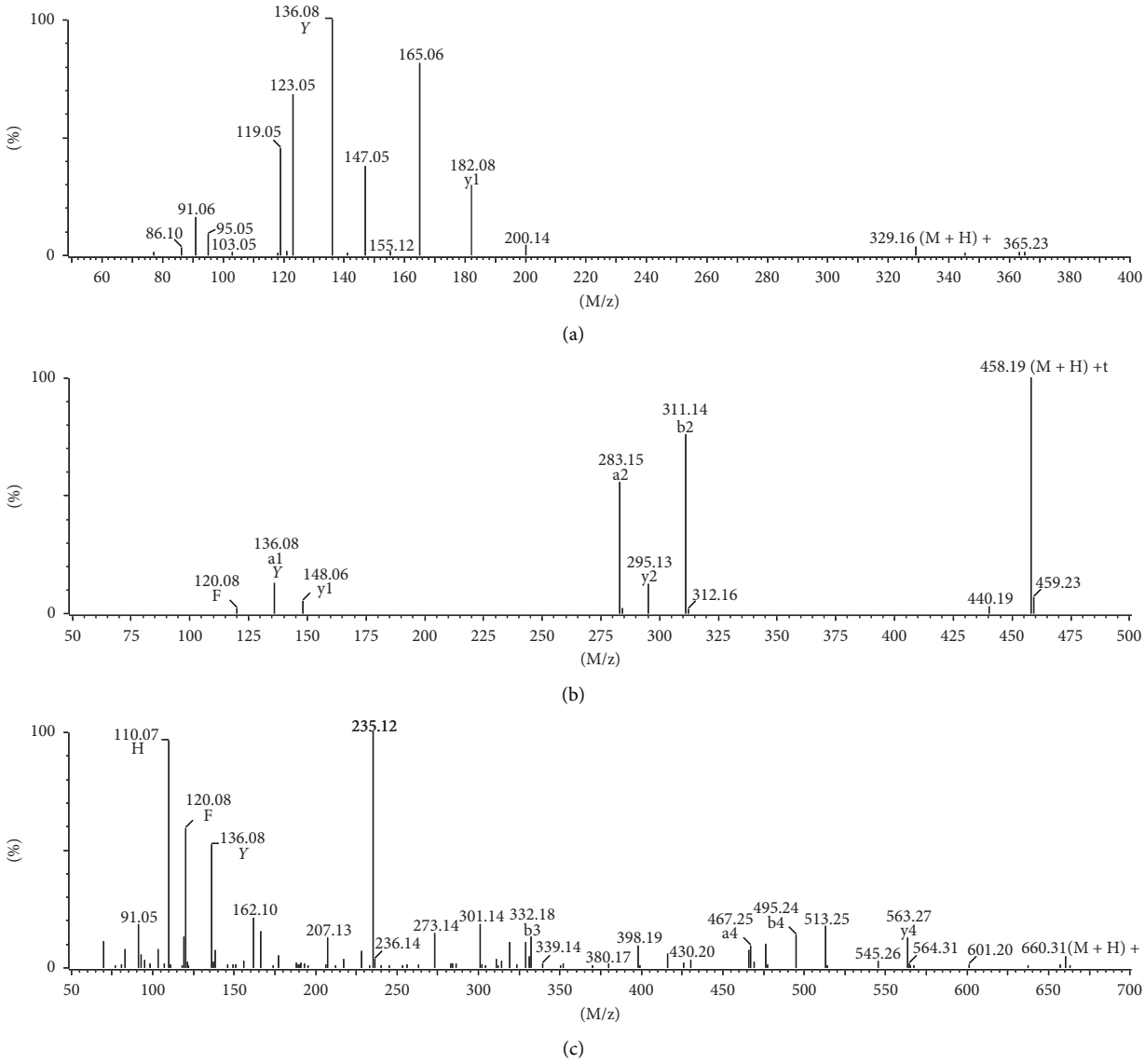


Figure 5: Tandem mass spectra of prominent peptides present in selected fractions. (a) Phe-Tyr. (b) Tyr-Phe-Glu. (c) Pro-Pro-His-Tyr-Phe.

which were identified by other researchers from PPH prepared using Alcalase [33] demonstrated the cleavage site in carboxyl side of Lys, Glu, and Phe. Although we did not identify the peptides TNKPVIF (f159-f167) and SNLAKSPE (f168-f1175), the antioxidant peptides SFDIK (f153-f158) and LDAK (f176-179) (Table 1) next to these two peptides were identified in our previous [29] and present works, respectively. The existence of Tyr-Phe-Glu in PPH which has been identified in our previous work [6] was also demonstrated in the present work again. Those results exhibited useful information for PPH's potential application in food industry and showed that enzymatic hydrolysis of potato protein by Alcalase is stable and could be repeated by different producers.

3.4. Effects of YFE on Lipid and Protein Oxidation of Caco-2 Cells. Since YFE was identified as the antioxidant peptide in PPH, the bioactivity of YFE on cellular antioxidation was involved. The cell viability of Caco-2 cells was higher than 85% when the concentration of YFE was 1 mg/mL. Hence, the YFE concentration of 1 mg/mL was used for the cellular oxidation trials. The oxidative stress induced by H_2O_2 was able to result in oxidation of lipid and protein in cellular membrane. The formation of MDA and carbonyl was used as the biomarker of Caco-2 cell oxidation, as shown in Figure 6.

Normal Caco-2 cells (NEG group) contained a basal level of MDA and carbonyl due to normal oxidative metabolism. After treating with 0.75 mm H_2O_2 for 4 h, the concentration of MDA and carbonyl groups of POS samples increased by 143% and 116% ($p < 0.05$), respectively. Those indicated the oxidative damage on cells. When Caco-2 cells were pretreated with YFE, MDA formation induced by H_2O_2 oxidation was reduced by 84.5% ($p < 0.05$). The formation of carbonyl groups was inhibited, and the concentration of carbonyl group changed a little ($p > 0.05$). The results suggested that YFE was able to suppress cellular oxidation

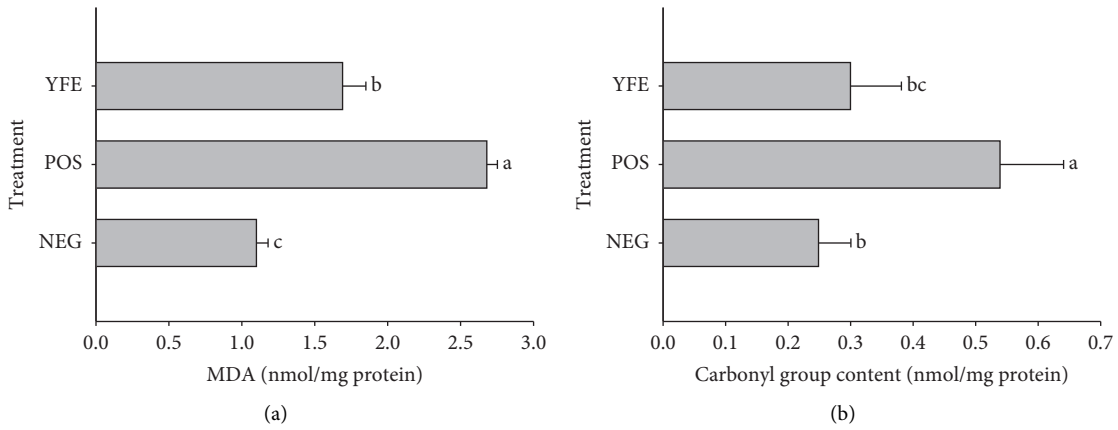


Figure 6: Effect of YFE pretreatment on lipid (a) and protein oxidation (b) of Caco-2 cells under the oxidation stresses induced by H_2O_2.

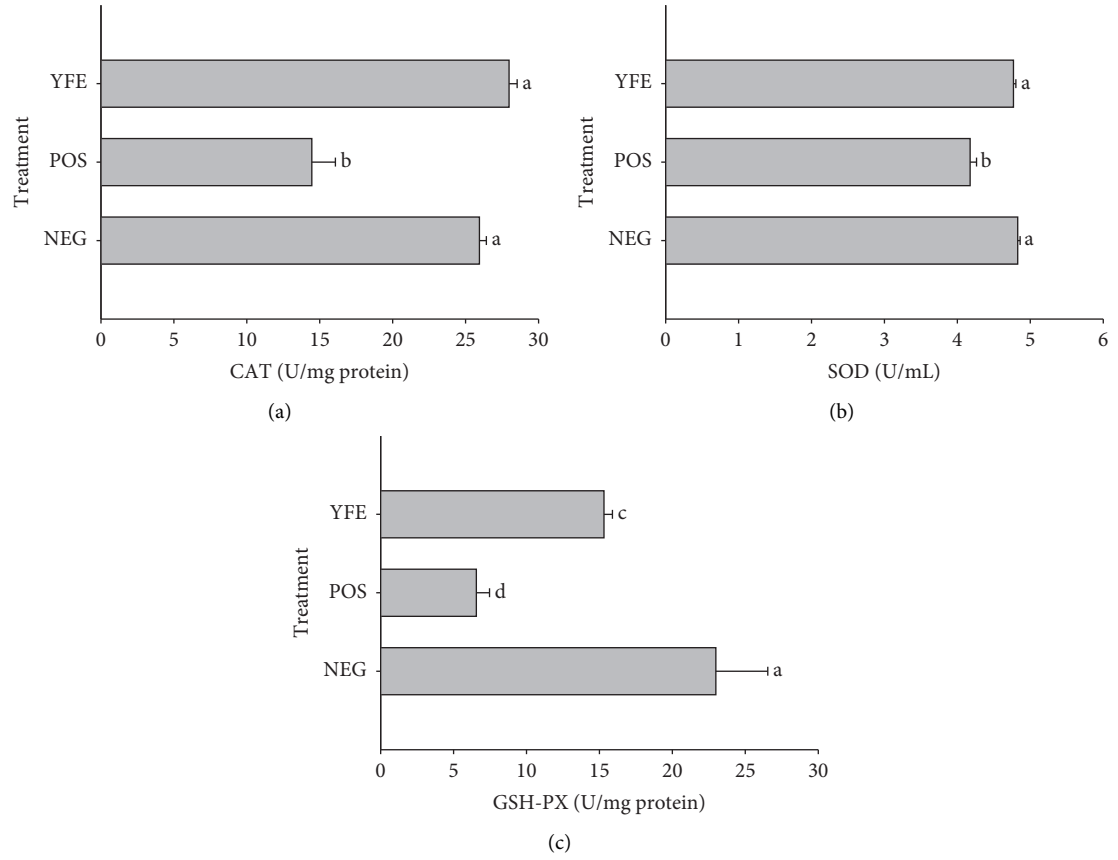


Figure 7: Effect of YFE pretreatment on the activities of antioxidant enzymes CAT (a), SOD (b), and GSH-PX (c) of Caco-2 cells under the oxidation stresses induced by H_2O_2.

induced by H_2O_2. These protective effects may be due to the radical scavenging activities of YFE or the activation of the cellular defense system against oxidative stress by YFE.

3.5. Effects of YFE on Antioxidant Enzyme Activities. The role of antioxidant enzymes such as CAT, GSH-PX, and SOD in the cellular antioxidant defense system was critical to elucidate the protective effects of YFE against oxidative stress in the cells. Those enzymes can repair cellular oxidation damage induced by free radical through electron-transfer reactions [34, 35]. The effect of YFE on the activities of CAT, GSH-PX, and SOD of Caco-2 cell is shown in Figure 7. H_2O_2 induced oxidation on Caco-2 cells reduced the activities of CAT, GSH-PX, and SOD of Caco-2 cells. Treating the Caco-2 cells with YFE was able to recover 100%

of the CAT and SOD activities and 66.6% of the GSH-PX activity. The activities of CAT, GSH-PX, and SOD in YFE treated cells increased by 93.5%, 133%, and 114% ($p < 0.05$) when compared with POS samples. Our results were similar to several previous results reporting that antioxidant peptides were able to protect cells against oxidation stresses induced by exogenous oxidants [36–39].

4. Conclusions

His, Phe, and Tyr were shown to be related to $ABTS^{+\cdot}$ scavenging activity of PPH by PCA. Some of the antioxidant peptides including Phe-Tyr, Tyr-Tyr, Tyr-Phe-Glu, and Pro-Pro-His-Tyr-Phe which were based on Phe and Tyr were identified by UPLC-MS/MS after fractionation and purification. The results indicated that multivariate analyses like PCA could be used to predict the possible sequence of antioxidant peptides before identification. The antioxidant peptide Tyr-Phe-Glu was able to suppress Caco-2 cellular oxidation induced by H_2O_2 through improving the activities of antioxidant enzymes including CAT, GSH-PX, and SOD.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

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Supplementary Materials

Figure S1: cumulative contributing rate of principal component. Table S1: amino acid composition of fractions of PPH isolated using gel filtration and ammonium sulfate precipitation. (*Supplementary Materials*)

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Review Article

Characterization of Novel Edible Films and Coatings for Food Preservation Based on Gum *Cordia*

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As a pharmaceutical component, gum *Cordia* has been applied to improve crop resistance against many diseases. A large amount of gum appears around the fruit after soaking in an aqueous system. The mucilage possesses outstanding technofunctional properties as an emulsifier, thickening agent, and binding and stabilizing component in food and drug industries. The backbone of gum Cordia is composed of (1-2)-linked L-arabinofuranosyl and (1–6)-linked D-glucopyranosyl residues. This manuscript reviews the technofunctional properties and applications of gum *Cordia* in food systems. Particularly, our focus has been given to its application as a natural source for the formation of edible films and coatings for increasing the shelf life of food products and for the food preservation as a potential ingredient in formulation. The future research perspectives are also highlighted.

1. Introduction

Nonbiodegradable and synthetic polymeric substances are vastly used as food packaging materials that have caused significant environment concerns all over the world. Possible alternatives for plastic packaging materials are those obtained from natural and renewable resources such as polysaccharide- and protein-based films and coatings [1–4]. These films and coatings have no effect on the manufacturing process of food or its ingredients and can be readily applied as thin biopolymer layers through a wrapping or submerging process. Biopolymer-based films and coatings can be loaded with natural antioxidants and antimicrobials and other bioactive components and flavors. They are also edible, available, and biodegradable which make them a better option in comparison with synthetic materials [5, 6].

In contrast to synthetic and semisynthetic polymers, fruit polysaccharides are for sure vastly available, cheap in processing costs, nontoxic, biodegradable, and not dangerous to the environment, and these features make them to be applied more rather than the synthetic agrochemicals [7]. Consequently, one of the less utilized and remarkable fruits, *Cordia* with different species, could be a novel and potential plant source of hydrocolloids. Noteworthy, physicochemical properties of natural polymers have made them adorable and applicable for different applications in pharmaceutical and food industries [8]. *Cordia* is a flowering plant genus from the Boraginaceae family. This genus with almost 250 species generally grows in warm regions. *C. latifolia*, *C. myxa*, *C. dichotoma*, and *C. abyssinia* are the four species of *Cordia* which are considered advantageous for a number of applications in different scientific articles [9]. *Cordia* is found in tropical and subtropical zones of Africa, America, Oceania, and Asia [10]. The structure of gum *Cordia* is formed from the backbone of (1-2)-linked L-arabinofuranosyl and (1–6)-linked D-glucopyranosyl residues inside arabinoglucan formation [10]. Gum *Cordia* affects health positively as it has valuable nutritional compounds. Some healing aspects of gum *Cordia*

Table 1: Constitution contents and pH amounts related to gum *Cordia*.

Chemical composition content (%)							
Moisture	9.26 [10]	9.10 [15]		9.1 [16]	8.34 [17]		6.90 [7]
Protein	2.47 [10]	2.60 [15]		2.6 [16]	12.75 [17]		8.90 [7]
Total carbohydrate	78.22 [10]						77.51 [7]
Fat							1.00 [7]
Ash	1.75 [10]	0.70 [15]	6.58 [12]	0.7 [16]	0.98 [17]	1.47 [18]	5.86 [7]
Soluble sugar							
Uronic acid		8.7 [15]		8.7 [16]	10.22 [17]		
pH and yield							
pH			6.5 [12]			7.2 [18]	
Yield (%)				1.2 [16]	1.56 [17]		
Mineral ion content (ppm)							
Calcium							8700 [7]
Phosphorus							700.21 [7]
Potassium							4.40 [7]
Zinc							186.11 [7]
Iron							120.97 [7]

involve anti-inflammatory, astringent, antimalarial, diuretic, febrifuge, anthelminthic, cicatrizant, cough suppressant, and appetite suppressant properties and is capable of curing lung diseases, leprosy, and urinary infections. Moreover, some studies show other applications of gum *Cordia* such as the sustained-release matrix in the bead, nanoparticles, tablets, and microcapsules, and also utilization as an emulsifier and binder [11, 12].

This manuscript reviews the technofunctional features of gum *Cordia* in food systems. However, the main focus has been given to its application as a natural polymer for the formation of edible films and coatings to increase the shelf life of food products.

2. Gum *Cordia*

2.1. Chemical Constitution. Purity is probably the first item for identifying the properties of gums. Normally, gum *Cordia* includes 77–78% carbohydrate, 2.6–12.75% protein, 6.90–9.26% moisture, 0.7–5.86% total ash, and 0-1% fat (Table 1). Carbohydrate is obviously the most abundant constituent in gum *Cordia*. It is more than the reported amount for gum ghatti (78.36%) and guar gum (71.1%) and close to the amount in the locust bean gum (85.1–88.7%) [13, 14]. The subtraction of other component contents of the gum from 100 equals 77.51% carbohydrate which is to a slight extent less than the total carbohydrate content acquired by the phenol sulfuric acid assay which equals 84.32%. Insufficient ashing (550°C and 3 h) might be the reason for this distinction. This amount of carbohydrate shows the high purity of gum *Cordia*. In addition, besides its purity, this gum has a good extraction efficiency that makes it a possible candidate for replacement of some commercial gums [7].

Functional properties such as stabilizing, emulsifying, and film-forming abilities could be due to the protein content inside the gum [19]. The protein fraction of gum *Cordia* was similar to that of the guar gum (8.19%) and higher than that of the locust bean gum (5.2–7.4%) and xanthan (2.125%) [20]. Although more studies are needed, it can be considered that this gum is an emulsifier and can decrease surface tension since it has a high level of protein. The percentage of ash in gum *Cordia* was considerably less than that of the guar gum (11.9%) and close to that of the xanthan gum (1.5%), locust bean gum (0.7–1.5%), and gum Arabic (1.2%) [20]. It is indicated by the obtained results that the water soluble part of ash inside gum involves 3.64% of the whole ash amount which is 5.86% [7].

Methyl esterification of some uronic acid groups of gum *Cordia* was carried out in both ethanol and acid precipitation to 38% methoxyl content [16]. Functional features of the polysaccharide, including formation of gel, are probably affected by methoxyl groups due to the investigation of low and high methoxy pectins [21]. No starch was observed as no sample turned blue during the iodine test. This polysaccharide was almost insoluble in organic solvents and soluble in hot water with the appearance of a colloidal solution. It also had near-neutral pH in the form of 1% (w/w) solution [18]. Additionally, the solution of gum *Cordia* has the pH of around neutral as well. That possibly means no irritative impact to the mucous membrane and epithelium is expected from gum *Cordia* in the gastrointestinal tract [22].

The mineral compounds in hydrocolloids have an impact on functional and physicochemical behaviors. For instance, the trace amount of iron (Fe) is destructive to the emulsifying ability of hydrocolloids [23]. Furthermore, gel-forming capacity and rheological properties of polyelectrolyte hydrocolloids can be influenced by the calcium ion (Ca^{2+}) [24]. Cadmium and some other ions with significant concentrations can cause health problems. Gum *Cordia* consists of 19700 ppm potassium, 8700 ppm calcium, 700 ppm phosphorus, 186 ppm zinc, and 120.97 ppm iron, but Ca and Cu concentrations did not reach the method detection limits (MDL). Table 1 shows the measured amounts of minerals. It can be found from these results that gum *Cordia* is rich in potassium (19700 ppm), calcium (8700 ppm), and zinc (185.93 ppm) though its copper and cadmium amounts are negligible. Gum *Cordia* has more valuable nutritional compounds than other commercial gums, such as guar, gum Arabic, and xanthan [25].

Table 2: Ratio of sugar composition in gum Cordia.

	Haq et al. [9]	Benhura and chidewe [15]
Arabinose	11	9
Galactose	31	27
Glucose	18	10
Mannose	11	17
Rhamnose	17t	21
Uronic acid	6	5
Xylose	6	11

Therefore, in pharmaceutical and food science fields, gum *Cordia* could certainly be known as a value-added byproduct.

2.2. Carbohydrate Analysis. HPLC method was used to study sugar hydrolysates from gum *Cordia*. Arabinose, galactose, glucose, mannose, rhamnose, uronic acids, and xylose were traced, and in two different studies, respectively, 11 : 31 : 18 : 11 : 17 : 6 : 6 [9] and 9 : 27 : 10 : 17 : 21 : 5 : 11 [15] ratios were obtained. Table 2 displays the results of these analyses. As a result of inadequate digestion, it was impossible to report the absolute mass basis of hydrolysates. The data from these two studies cannot be concluded, as digestion was insufficient, and they were unlike the mentioned ratios of *C. abyssinica*.

2.3. Molecular Information. Functional properties of gum are remarkably affected by its molecular weight [26]. In gel permeation chromatography (GPC), gum *Cordia* presented a major peak, after which two small peaks appeared. Consequently, it is estimated that the formation of gum *Cordia* consists of three parts with different molecular weights. Haq et al. [9] observed two peaks related to gum by the GPC method, showing the average molar mass of 1.81×10^6 and 3.89×10^3 g·mol^{-1}. Usually, the average molar mass of the first fraction in gums with a plant base is about 10^5 g·mol^{-1}, which is almost lower than the amount for gum *Cordia*. Gum *Cordia* has the average molecular weight of 2.23×10^4 g·mol^{-1} [7] that is lower than plenty of commercialized gums such as xanthan with 4.05×10^6 g·mol^{-1} [27], gellan gum with 1.64×10^6 g·mol^{-1} [28], and guar gum with 1.45×10^6 g·mol^{-1} [29]. The number average molecular weight (Mn) of gum *Cordia* was estimated as 1.34×10^4 g·mol^{-1}. A molecular factor for understanding the molecular weight distribution and homogeneity of biopolymers is the polydispersity index (PDI = Mw/Mn), which often ranges from 1.16 to 2.86 in natural polymers. Gum *Cordia*, with a value of 1.66 [7], had a lower PDI than lots of hydrocolloids since more uniformity was found in the gum, and on the contrary, was near to that of gum Arabic with 1.2652 and guar gum with 1.21 [30]. Unlike other gums with a plant base, such as gum Arabic (PDI = 2.27) and kappa-carrageenan (PDI = 3), both mentioned fractions of gum *Cordia* had PDI of around a uniform condition value [9]. PDI expresses the heterogeneity inside chains of the polymer. Molecular mass distribution of gum *Cordia* is very limited, so the PDI value is about 1. This is a desirable trait of gum that makes it favorable to be used in edible films since better tensile strength occurs in chains with homogenous structures.

2.4. Fourier-Transform Infrared Spectroscopy (FTIR). Analysis of gum *Cordia* by FTIR spectrometry was carried out for recognizing its functional groups. As a matter of fact, all of the usual peaks and bands related to polysaccharides can be seen in the gum spectrum [7]. At 848.2 cm^{-1}, a noticeable shoulder represents the linkage of alpha and beta in the structure of gum [23]. Finger print area (from 1000 to 1200 cm^{-1}) is a region in which any polysaccharide shows a particular behavior. This area explains the distinctions in structures of biopolymers. The signal at 1030 cm^{-1} is related to uronic acid. There are some peaks in the range of 950 to 1200 cm^{-1} as a result of the stretching vibration associated with alcoholic CO in COH bands inside carbohydrates [22]. The observed peak at 1635 cm^{-1} shows valence vibration and carboxyl groups. Gel-forming ability and viscosity could be affected by possible interactions between some ions, such as calcium and carboxyl groups [31]. Carboxylic ester causes the wavenumber near 1737 cm^{-1}. The small peak at 2921.91 cm^{-1} expresses the stretching vibration of C-H related to the methylene group. Double overlapping with hydroxyl groups might cause this peak as well. The peak at 3438.81 cm^{-1} is related to the stretching vibration of O-H. Hydrogen bonding of the hydroxyl groups inside glucopyranose rings is also associated with this enormous peak [32].

2.5. Differential Scanning Calorimetry (DSC). Differential scanning calorimetry (DSC) uses temperature for tracking the obtained or lost heat in samples, during chemical and physical changes. Impurities are explained by wide asymmetric curves which could also be the sign of two or more thermal processes, and relative purity is described by an obvious symmetric peak of the melting endotherm [18]. Phase transitions in polymers are commonly investigated by DSC since it is a very precise and sensitive method. Occurrence of changes in the main chain during heating causes two endothermic behaviors in the DSC thermogram of gum *Cordia*. The first endothermic reaction of gum *Cordia* happened as a result of bound and free water loss, and a vast peak appeared around 147°C which is the glass transition temperature (T_g). Decomposition or melting of gum at 238°C (melting temperature or T_m) brings up the second endothermic reaction and provokes a little peak at the related temperature. It can be found that although gum *Cordia* looks crystalline, it has an amorphous characteristic, considering its wide DSC thermogram. In the industry (production and storage stages), it is important to have information about the glass transition temperature and the items that have impact on it, such as additives and moisture that change the formation of molecules and consequently simplify the transformation to the crystalline or rubbery phase [32]. In different studies, low crystallinity was observed for substances with a low glass transition temperature [22].

2.6. X-Ray Diffraction (XRD). Remarkable peaks of gum *Cordia* appear of the order of 2Θ which equals 26°, 29°, 30°, and 5°. Meanwhile, uncertain and very small peaks and also shoulder forms stuck to other strong peaks can be seen. XRD data estimate the occurrence of both crystalline and amorphous forms in gum *Cordia*, which confirms the results of DSC [18].

3. Applications

3.1. Biodegradable Edible Films. The quick increase in the production rate of nondegradable petroleum-based plastic materials causes serious environmental concerns [33]. Therefore, many efforts have been made to find environmentally friendly materials. Natural polymers (proteins and polysaccharides) have received much attention due to biodegradability [17]. One of the important applications of gum *Cordia* is the production of edible films. Haq et al. [34] studied the properties of the edible gum *Cordia* film with added beeswax. Beeswax affected the film by decreasing its elongation at break, tensile strength, and Young's modulus. Gum *Cordia* films with beeswax had a water vapor permeability of $0.06–0.33 \times 10^{-10}$ g·m^{-1}·s^{-1}·Pa^{-1}, which is lower than that of the control films. Additionally, it is found that oxygen permeability becomes greater from 0.39 to 18.73 g·m^{-1}·s^{-1}·Pa^{-1} in a beeswax concentration of 0.05 g·g^{-1}. Nevertheless, oxygen permeability stopped increasing as beeswax reached higher concentrations. Results obtained from this work will draw attentions to *Cordia* as a plentiful source of gum and ease its applications. Further progression is also expected in edible packaging made from gum *Cordia*, for fresh material coating, specifically. Haq et al. [35] analyzed the attributes of the edible gum *Cordia* film affected by plasticizers. Edible gum *Cordia* films were made considering the concentration and kind of the plasticizer and then their permeability to gas, and also their rheological and thermal characteristics were studied. The solution casting method was carried out for production of the films. Chosen plasticizers were PEG (polyethylene glycol) 200, PEG 400, sorbitol, and glycerol for an amount of 0.1 to 0.3 g·g^{-1} dry polymer weight basis. It was indicated that film behaviors rely on the concentration and nature of the plasticizer. Gum and plasticizers had interactions which were recognized by FTIR spectroscopy. Miscibility of gum and plasticizers was estimated by DSC. The glass transition temperature was observed inside the range of −66 to −11°C. From the rheological point of view, these films had elongation at break of more than 10% and tensile strength of higher than 10 MPa. Glycerol, sorbitol, PEG 200, and PEG 400 caused the most remarkable tensile changes, respectively. Water vapor permeability was measured from 0.91 to 5.5×10^{-10} g·m^{-1}·s^{-1}·Pa^{-1}. Oxygen permeability was obtained in range of 0.16 to 5.31×10^{-15}·g·m^{-1}·s^{-1}·Pa^{-1}. Edible film could be prepared by the casting method from gum *Cordia* added with various plasticizers. Modification of these films is possible by using different plasticizers and their various concentrations. Miscibility and interactions between plasticizers and gum were observed in the produced films. Addition of glycerol, sorbitol, PEG 200, and PEG 400 to gum *Cordia* films, respectively, was responsible for higher induced elongation at break and tensile strength. Adding PEG 400 can also hold the oxygen permeability in a lower amount. This research announces *Cordia* as a plant with simple uses in the mentioned area and gives hope for improvement of edible packaging. Haq et al. [36] reported that plasticizers have an impact on optical and sorption characteristics of edible gum *Cordia* films. The goal was to identify the biodegradable film made from the extracted gum of plant *Cordia*. PEG200, PEG 400, sorbitol, and glycerol were used as plasticizers with concentrations of 0 to 30% to investigate their influence on optical and sorption behaviors of the casted film. The results of this analysis corresponded to the type II isotherm, according to the GAB model of sorption since the equilibrium moisture content of films increased by increasing the concentration of plasticizers. Increasing the concentration of the plasticizer also increased the value of the monolayer, as the most effective one was glycerol with 0.93 g·g^{-1}. Total color difference (ΔE) was enhanced as more plasticizers were added, and glycerol had the most impact again. All samples resisted 200 to 280 nm wavelength of UV spectra. A comparative film with more desirable characteristics for edible coating applications could be made from *Cordia myxa* fruit gum. Plasticizers along with anionic *Cordia* gum create the film. In comparison with typically used edible films, this one involved more preferable optical properties and sorption isotherms. Each plasticizer was tested in terms of its concentration to indicate its efficacy, and it became clear that glycerol was the most effective. By applying the GAB equation, sigmoidal curves of isotherms related to gum *Cordia* films could be drawn. Water molecules bond to sorption sites of gum by a lower rate since plasticizers reduce the needed energy for this action. The total color of the gum *Cordia* film was also lowered by presence of plasticizers. Incorporation of gum *Cordia* with every plasticizer produced transparent films. But, the UV spectrum could not pass through these films easily.

3.2. Coating Applications. Application of gum *Cordia* as a coating especially for nuts was reported in various research studies. El-Mogy et al. [37] investigated the impact of gum *Cordia* on the postharvest quality of fresh artichoke bottoms. Results showed that the addition of ascorbic acid or $CaCl_2$ to gum *Cordia*-based edible coatings improves the amount of total phenolic compounds and vitamin C and prevents the weight loss. Gum *Cordia* incorporated with ascorbic acid considerably inhibited polyphenol oxidase and browning activities. Gum *Cordia*-free $CaCl_2$ solution reduced the count of psychrotrophic and mesophilic bacteria. The strongest treatment against *E. coli* and molds was the combination of $CaCl_2$ and gum *Cordia*. Therefore, a mixture of gum *Cordia* and $CaCl_2$ was suggested as a proper coating for artichoke bottoms during postharvest storage. More research studies are needed for the commercial application of gum *Cordia* in preserving fresh-cut artichoke bottoms. Haq et al. [38] studied potentials of gum *Cordia* to prevent oxidation of lipids in peanuts as an antioxidant carrier.

Attachment to the polymer matrix (e.g., edible films with a gum base) enhances the efficiency of antioxidants. Good emulsification and adhesion capabilities of gum *Cordia* make it a possible carrier for antioxidants. The purpose of this investigation was to inspect the capacities of gum *Cordia* for coating peanuts in the form of an antioxidant carrier. Antioxidants for delivery action included vitamin C, BHA (butylated hydroxyanisole), and BHT (butylated hydroxytoluene) used in a comparison between gum *Cordia* and carboxymethyl cellulose as carriers. Carrier efficiency of two gums was compared by analyzing the oxidized flavor, thiobarbituric acid-reactive species, and peroxide value of uncoated and coated peanuts which were kept at 35°C for 126 days. Uncoated and coated peanuts showed significant differences ($p < 0.05$). Gum *Cordia* was a better carrier in antioxidant delivery. According to the peroxide value (40°meq of $O_2\,kg^{-1}$), gum *Cordia* with BHA/BHT (290% more shelf life than the uncoated sample), gum *Cordia* with BHT (244%), gum *Cordia* with BHA (232%), carboxymethyl cellulose with BHA/BHT (184%), carboxymethyl cellulose with BHA (139%), carboxymethyl cellulose with BHT (119%), gum *Cordia* with vitamin C (96%), and carboxymethyl cellulose with vitamin C (46%) fulfilled carrier responsibility, respectively. Therefore, it is clear that gum *Cordia* acts as a capable carrier for antioxidants and probably other functional stuffs in the form of edible coating. Other conditions are being studied for more advances in using this gum as a coating film. Abdul Haq et al. [39] carried out assessments to improve the shelf life of *Pinus gerardiana* by gum *Cordia* edible coating. Anionic gum of *Cordia* is the reason for its good adhesion ability. *Pinus gerardiana* is full of unsaturated fatty acids which makes its unshelled nuts vincible to oxidation. Carboxymethyl cellulose and gum *Cordia* were compared in terms of preventing rancidity of *Pinus gerardiana* in the form of edible coatings. Carboxymethyl cellulose and gum *Cordia* each included a free sample and a sample containing natural antioxidants. Studied antioxidants were α-tocopherol and the methanolic extract of *Cordia myxa*. Sensory and chemical analyses were carried out on all samples (coated and uncoated) which were kept at 35°C for 112 days. Uncoated and coated nuts demonstrated significant differences ($p < 0.05$). In accordance with the peroxide value (20 meq of $O_2\,kg^{-1}$), samples involving gum *Cordia* with the added extract of *Cordia myxa*, carboxymethyl cellulose with the added *Cordia myxa* extract (circa 60%), free gum *Cordia* (circa 25%), and free carboxymethyl cellulose (circa 15%) showed the most effectiveness, respectively. α-Tocopherol did not exhibit any impact on nuts. Consequently, gum *Cordia* could act as a potent natural edible film for inducing higher shelf life in pine nuts. This gum might also be an option in other foods for increasing shelf life and invincibility to rancidity by its coating capability. Haq and Hasnain [40] studied gum *Cordia* coating with involvement of antioxidants to prevent rancidity of peanuts. Abilities of two gums, i.e., gum *Cordia* and carboxymethyl cellulose, in improving shelf life of peanuts were compared. Samples of both free and with natural antioxidants of carboxymethyl cellulose and gum *Cordia* were investigated. α-Tocopherol and the methanolic extract of the *Cordia myxa* fruit were the present antioxidants. Peanuts were held at 35°C for 126 days, and their sensory and chemical properties were analyzed. By considering the peroxide value (40 meq $O_2\,kg^{-1}$), gum *Cordia* having the *Cordia myxa* extract showed 122%, free gum *Cordia* showed 91%, carboxymethyl cellulose having the *Cordia myxa* extract showed 80%, and free carboxymethyl cellulose showed 70% growth in shelf life of samples. No beneficial effect was induced by α-tocopherol. It is estimated that increasing shelf life of peanuts is possible by gum *Cordia* coating although no information exists about its mechanism of action. More studies are being done for a better understanding of the physicochemical attributes of edible gum *Cordia* coatings. Foods with a high lipid content could be the targets of this natural coating for increasing their shelf life.

4. Conclusion

This review article, which is based on the findings of the studies on gum *Cordia*, demonstrates that the gum *Cordia* may be used to improve the viscoelastic, textural, stability, and other parameters of foods and drugs. It can be applied to create novel applications such as encapsulation and delivery of bioactive ingredients. These beneficial properties suggest this gum as a proper component which could potentially be used for different purposes in pharmaceutical and food products. But, still more research is needed to find the chemical structure and other important technofunctional characterization of gum *Cordia*. This gum may be of technological importance and can be used for the improvement of food product quality.

More research works are required to be carried out in the field of modified processing methods and isolation and purification techniques. Structure-function relationship elucidation and backbone-directed chemical modification of gum *Cordia* might expand its applications in the food industry.

Data Availability

The data used to support the study are included within the article. Any more information can be obtained by contacting the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

The Effects of Gum Cordia on the Physicochemical, Textural, Rheological, Microstructural, and Sensorial Properties of Apple Jelly

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The aim of this research was to study the effects of gum cordia on the physicochemical, color, textural, rheological, microstructural, and sensorial properties of apple jelly. Apple jelly was prepared by replacement of 0, 25, 50, 75, and 100% of pectin with gum cordia. The results showed that gum cordia had a significant effect on the physicochemical properties (ash, protein, TPC, DE, and color) of apple jelly. The total phenol content of the sample significantly increased with the addition of gums. The rheological properties showed that a sample containing 75% gum cordia was similar to control and had the highest apparent viscosity, loss moduli (G''), storage moduli (G'), and complex viscosity. Also, the sensorial properties showed that a sample containing 75% gum cordia had a high score in texture, taste, appearance, and overall acceptability. The results suggested that gum cordia as a polymer can be successfully employed for the formulation of jelly for improving technofunctional properties of jelly.

1. Introduction

Gum cordia is an anionic polysaccharide containing uronic acid and it is extracted from *Cordia myxa* fruit [1]. This gum has some technofunctional properties such as good emulsifying and binding properties [2]. Also, the application of this gum as a novel nanoparticle for drug delivery has been evaluated [3]. Some other applications including production of paper and cardboard were reported previously [4]. Haq et al. [4] studied the effects of gum cordia as an antioxidant carrier for improving shelf life of nuts. Gum cordia has pseudoplastic behavior with a high viscosity so its strong emulsifier, thickener, and stabilizer in food industry and pharmaceutical technology [5].

Today, there is a lot of research on the application of fruits for improving body health. They reported fruit consumption reduced obesity, diabetes, and cardiovascular disease. The health properties of fruits are related to the high content of antioxidant ingredients such as phenolic acids and vitamins. On the other hand, the shelf life of some fruits is very low and they are perishable, so researchers try to convert fruits to some products such as jelly and jam. Jelly is a product containing fruit juice, gelator, sugar, and acid and it has a firm structure [6]. Apple fruit is a good source of nutritional ingredients such as phenolic acids, organic acids, minerals, and reducing sugars [7]. Also, it is a rich source of vitamins including ascorbic acid [8] and it has antioxidant ingredients [9].

The first production of jelly was based on the high-methoxyl pectin and it was a firm gel with 2.8–3.5 pH and containing 55% soluble solid components. pH was a very important parameter in the production of gel in the conventional jelly due to the high application of pectin. The production of jelly with low methoxyl pectin needs some other ingredients such as bivalent ions. Today's application of hydrocolloids for production of jelly was increased. Some main parameter of hydrocolloids such as creating viscosity, texture, and structure in final products are important. Hydrocolloids can hold water in their structure and improve viscosity and gel structure [10–12]. One of the attractive

fields in the production of jelly is the application of a mixture of hydrocolloids in the jelly and researchers try to improve functional properties of products with a combination of pectin and hydrocolloids [13, 14].

The aim of our research was to determine the effects of gum cordia on the physicochemical (moisture, protein, ash, carbohydrate, fiber, sugar, total phenolic content, pH, and DE), color (L, a, b, ΔE and YI), rheological (viscosity and rheometry parameters), and sensorial properties of apple jelly.

2. Material and Methods

2.1. Material. Apples were bought at a local market (Shiraz, Iran) and stored at 5°C during 48 h prior to juice extraction. Pectin, HM (green ribbon) which is high-methoxyl, pectin (DE ~ 60%), was obtained from Obipektin (Bischofszell, Switzerland). All other chemicals were bought from Merck (Darmstadt, Germany).

2.2. Gum Extraction. The extraction of gum from fruit was done by the method described by Haq et al. [4] with some minor modifications. Fruits were added to hot double distilled water (85°C) containing 1000 ppm of sodium metabisulfite in 1 : 4 ratio and stirred for 35 min. The suspension was filtered completely by a cloth. The separated liquid was centrifuged at 6000 g for 50 min. The gum was precipitated from clear supernatant by application of HCl in 1 : 100 ratio. Finally, the mixture was centrifuged at 3000 g for 15 min. The precipitate of the sample was washed with double distilled water for 3 times. The extracted gum freeze-dried and then completely ground to 60 mesh sieve and kept at 5°C until experiments.

2.3. Jelly Preparation. Apple juice was produced by hand peeling of apples and extracting with a juice maker. The main components of apple jellies were apple juice (900 mL), sucrose (80 g), and gelator (20 g). To create a good texture by pectin and setting pH, citric acid (2.7 g) was added. The best pH value for creating a good texture by pectin is 2.9 to 3.1, so pH of samples was set at 3. Apple juice, 60 g of sugar, and citric acid were homogenized and heated at 85°C. Then, gelator and 20 g sugar was homogenized and added to the suspension at the end of jelly cooking. Apple jelly suspension was heated to final soluble solids of jelly arrived at 66.8%. The time of heating was around 0.5 h. Final products were poured into the cups and kept at 5°C. Apple jellies were produced by replacement of 0, 25, 50, 75, and 100% of pectin with gum cordia as a gelator.

2.4. Chemical Composition Measurement. The moisture content of the sample was measured by the application of vacuum oven drier at 70 vacuum oven [15]. Ash, protein, carbohydrate, and fiber content were determined based on AOAC standard method [16].

2.5. Sugar Content Measurement. The percent of total reducing sugars was measured by the application of Fehling's A and B solution methods. The methylene blue was used as an indicator [17].

2.6. Total Phenol Content (TPC) Measurement. TPC of jellies was determined based on the method of Kopjar et al. [6] with some minor modifications. First, 200 mg of sample and 1.8 mL DDW were completely mixed, and then 10 mL of Folin–Ciocalteu reagent (10% w/w) and 8 mL of sodium carbonate solution (7.5% w/w) were added to it. The suspension was kept at 25°C for 120 min in a dark condition (to complete reaction). The absorbance of sample and control was measured by a UV-spectrophotometer at 764 nm. Gallic acid was used as a standard and our results were reported based on μg of gallic acid per g of jelly ($\mu g \cdot GAE \cdot g^{-1}$).

2.7. pH Measurement. For the determination of the pH value of samples, 1 g of jelly was homogenized with 9 mL DDW at 51°C using a mixer. The pH value of the sample was determined by a pH meter (OHAUS, Switzerland) [18].

2.8. Degree of Esterification Measurement. A titration technique based on the modified method of Pinheiro et al. [19] was used for the determination degree of esterification (DE). 0.4 g of dried sample wetted with ethanol in a bottle, and it was mixed with 40 mL DDW at 37°C. Samples were stirred for 120 min to completely dissolve. The resulting suspension neutralized by NaOH (100 mM) and the volume of NaOH was recorded (A) (in this part phenolphthalein was used as an indicator). Then, 20 mL of NaOH solution (100 mM) was mixed with samples and kept in 25 C for 120 min to saponify the esterified carboxyl groups of the samples. Then, 20 mL HCl (100 Mm) was added and mixed. Finally, titration of samples was done by NaOH (100 mM) and the volume of it was recorded (B). Esterified carboxy groups number was determined based on the following equation:

$$\mathrm{DE} = \frac{\mathrm{B}}{(\mathrm{B} + \mathrm{A})} 100. \quad (1)$$

2.9. Color Parameters Measurement. The surface color parameters of samples were determined using the CIELAB system (CR-410, Japan). $L*$, $a*$, $b*$, ΔE, and YI of samples were reported as the main parameters in jelly. The $L*$ (black (0) to white (100)), $a*$ (red (+100) to green (–100)), $b*$ (yellow (+100) to blue (–100)) indicate the color of samples. ΔE and YI were calculated based on Lab parameters [20].

2.10. Rheological Properties Measurement. Elastic (G'), viscous (G'') moduli, Tan δ, and complex viscosity of jellies at 25°C were measured by a plate geometry (diameter $d = 50$ mm, gap = 2 mm). Dynamic oscillatory test of jellies at angular frequencies (ω) from 0.628 to 62.8 rad/s in the linear viscoelastic range (LVR) at 0.5% strain. Rheological parameters were determined in a Paar Physica MCR 301

Table 1: The effects of pectin: gum ratio on the moisture, ash, protein, carbohydrate, and fiber content of samples.

	Moisture content	Ash	Protein	Carbohydrate	Fiber
P50G50	74.00 ± 0.58A	0.31 ± 0.01B	0.30 ± 0.05C	23.20 ± 0.69A	0.22 ± 0.01A
P75G25	76.30 ± 0.98A	0.36 ± 0.03C	0.23 ± 0.01B	23.00 ± 0.52A	0.20 ± 0.01A
P25G75	74.10 ± 1.61A	0.27 ± 0.02B	0.32 ± 0.04C	22.70 ± 0.87A	0.21 ± 0.05A
P100	74.50 ± 1.50A	0.43 ± 0.05D	0.18 ± 0.02A	24.87 ± 0.58B	0.22 ± 0.00A
G100	76.50 ± 1.50A	0.21 ± 0.01A	0.37 ± 0.04C	22.77 ± 0.58A	0.20 ± 0.01A

*Data represent the means of three independent repeated ± standard deviation. *Capital letters in each column showed significant differences ($P < 0.05$). *P50G50 (sample containing 50% pectin and 50% gum); P75G25 (sample containing 75% pectin and 25% gum); P25G75 (sample containing 25% pectin and 75% gum); P100 (sample containing 100% pectin) and G100 (sample containing 100% gum).

rheometer (Anton Paar GmbH, Graz, Austria), with controlled temperature (Viscotherm VT2, Phar Physica) [21].

2.11. Viscosity Measurement. The rheological behavior of samples was measured at 25°C and the shear rate range of 0.01–93.9 s^{-1} using an Anton Paar rheometer (MCR 302, Graz, Austria). Cone-plate geometry (type CP25-1) with 25 mm cone diameter and 0.052 mm gap size at 1° cone angle was used. System temperature was controlled by a Peltier system with an accuracy of 0.1°C. The apparent viscosity was determined at the shear rate of 19.1 s^{-1}.

2.12. Textural Properties. Textural features of the samples were investigated using a texture analyzer (Brookfield, USA) by a double compression test. 2.5 (diameter) × 1 (height) cm cylinder of each sample was compressed to 40% penetration depth with 5 mm/s pretest speed and 0.5 mm/s test speed using a cylinder probe of 25 mm diameter.

2.13. Microstructure. The surface morphology of the sample was analyzed by a scanning electron microscope (TESCAN Vega3, Czech Republic). Freeze-dried samples were covered with a thin layer of gold (Desk Sputter Coater DSR1, Nanostructural Coating Co., Iran). Micrographs were taken at an accelerating voltage of 20 kV [22].

2.14. Sensorial Properties Measurement. Sensorial properties of samples were determined based on five-point hedonic test described by [18] with some minor modification. Appearance, texture, taste, odor, and overall of jellies was evaluated from 1 (disliked extremely) to 5 (liked extremely) score. The sample was consumed by 10 semitrained student (male and female from 22 to 30 years) of the Department of Food Science and Technology of Kazerun Azad University, Iran. Jellies coded at random order and placed on plates with a glass of water. The study was done on a white bench under the usual light at 20°C.

2.15. Statistical Analysis. Averages and standard deviations of samples were reported based on the triplicate measurement. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used for comparing the averages at 5 percent significant level. The statistical analysis was done by SPSS 19 software (SPSS, IBM, Chicago, IL, USA).

Table 2: The effects of pectin: gum ratio on the total phenol content, pH, sugar content, and DE of samples.

	TPC	pH	Sugar content	DE
P50G50	26.80 ± 1.15D	4.21 ± 0.48A	21.00 ± 0.87A	83.70 ± 0.81B
P75G25	19.00 ± 0.52B	4.28 ± 0.28A	21.30 ± 0.75A	84.40 ± 0.58B
P25G75	23.30 ± 0.75C	4.08 ± 0.20A	20.40 ± 0.81A	82.20 ± 0.52A
P100	11.80 ± 1.04A	4.40 ± 0.06A	20.50 ± 1.15A	86.60 ± 0.64C
G100	34.70 ± 1.44C	3.90 ± 0.40A	22.60 ± 1.38A	81.80 ± 0.46A

*Data represent the means of three independent repeats ± standard deviation. *Capital letters in each column showed significant differences ($P < 0.05$). *P50G50 (sample containing 50% pectin and 50% gum); P75G25 (sample containing 75% pectin and 25% gum); P25G75 (sample containing 25% pectin and 75% gum); P100 (sample containing 100% pectin) and G100 (sample containing 100% gum).

3. Results and Discussion

3.1. Chemical Composition. The results of the chemical composition of apple jellies, including moisture, ash, protein, carbohydrate, and fiber content are reported in Table 1. Moisture content is a key quality factor in the packed food products such as jellies. It has some effects on the microbial quality and stability parameters of products [23]. Table 1 reports that there are no significant differences between the moisture content of samples. Mutlu et al. [18] reported that gelatin dose had not significantly ($P > 0.05$) influence the moisture content of different honey jelly candies. The presence of biopolymers has a significant effect on the water holding capacity of jelly samples [24]. The protein content of samples significantly increased with the addition of gum concentration. This is due to the protein content of gum cordia. The protein content of this gum was reported around 2 to 9% [25, 26]. The results showed that a sample containing 100% pectin had the highest carbohydrate content. The ash content of samples significantly decreased with the addition of gum concentration. There were no significant differences between the fiber content of the samples. The low changes in some composition ingredients are due to the differences between pectin and gum composition. Pectin is a pure ingredient but gum cordia contains protein and fiber.

3.2. Sugar Content, Total Phenol Content, pH, and DE. The results of TPC, pH, sugar content, and DE are shown in Table 2. The total phenol content of the sample significantly increased with the addition of gum concentration. There is some research that reported gum cordia had some phenolic

Table 3: The effects of pectin: gum ratio on the color properties of samples.

	L	a	b	ΔE	YI
P50G50	30.00 ± 0.58A	11.67 ± 0.33A	24.67 ± 1.33A	74.43 ± 0.51A	117.47 ± 4.95B
P75G25	31.33 ± 0.33AB	11.67 ± 0.88A	25.00 ± 0.58B	76.49 ± 0.22B	113.99 ± 5.11B
P25G75	29.33 ± 1.45A	12.33 ± 0.33A	23.33 ± 0.67A	73.82 ± 1.38A	113.63 ± 5.29B
P100	33.67 ± 0.88B	10.67 ± 1.20A	23.00 ± 0.58A	77.23 ± 0.66B	97.58 ± 3.64A
G100	28.00 ± 0.58A	12.33 ± 1.20A	26.00 ± 1.00B	73.23 ± 0.72A	132.65 ± 5.69C

*Data represent the means of three independent repeats ± standard deviation. *Capital letters in each column showed significant differences ($P < 0.05$). *P50G50 (sample containing 50% pectin and 50% gum); P75G25 (sample containing 75% pectin and 25% gum); P25G75 (sample containing 25% pectin and 75% gum); P100 (sample containing 100% pectin) and G100 (sample containing 100% gum).

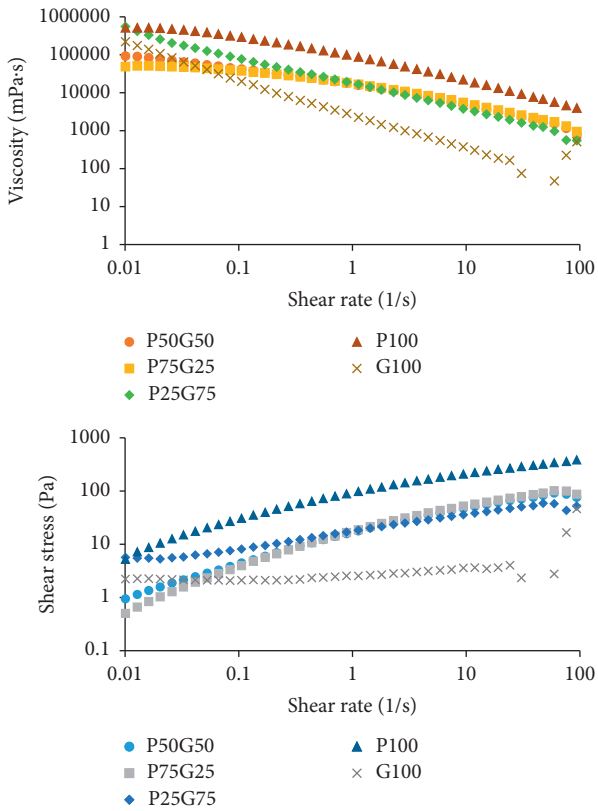

Figure 1: The effects of pectin: gum cordia ratio on apparent viscosity and shear stress of samples.

ingredients and had good antioxidant properties [25, 27]. The results showed that the replacement pectin with gum had no significant effects on the pH of samples. Also, the same results were reported by Kamal et al. [28]. They reported that addition gelatin has no significance on the pH of the jelly and the pH of all samples was around 4. The addition of gum had no significant effects on the sugar content of samples. DE of the sample significantly decreased with the addition of gum concentration. This is due to the higher degree of esterification of pectin.

3.3. Color. Color of food products is an important quality factor for consumer and usually its effects on the anticipation of people about quality and color parameters [29]. Table 3 reported the lightness ($L*$), redness ($a*$), yellowness ($b*$), color changes (ΔE), and yellow index (YI) of apple jelly samples as a main color parameter. Lightness ($L*$) of apple jelly samples was decreased after the addition of gum cordia (Table 3) which was due to the presence of pigments in the gum. Kamal et al. [28] reported that gelatin concentrations had significant effects on the decrease of the $L*$ value of jelly. The addition of gum had no significant effects on the Redness values ($a*$) of samples. Mutlu et al. [18] reported that gelatin concentration had no significant ($P > 0.05$) effects on the Redness values ($a*$) of jelly. Yellowness values ($b*$) were observed to increase with the addition of gum concentration. The results of ΔE showed that with the addition of gum concentration, ΔE was decreased. Also, YI of the sample showed that with an increase in gum concentration, these parameters were increased. Changes in color parameters are due to some pigments in the gum cordia [30].

3.4. Viscosity Properties. The results in Figure 1 show apple jelly samples had shear-thinning behaviors. The same results about shear-thinning behaviors of jelly were reported by Prakash et al. [31] and Figueroa and Genovese [21]. High molecular weight, the aggregated state, and hydrogen bonds between polymer chains are mainly responsible for the shear-thinning behavior of polysaccharides [32]. Shear-thinning properties of biopolymers is an important and key parameter in the food industry, during some food processing such as pumping and filling; these properties are very useful. The higher viscosity at lower shear rates leads to a desirable mouthfeel during mastication [33]. The apparent viscosity of P50G50, P75G25, P25G75, P100, and G100 at the shear rate of 19.1 s^{-1} and 25°C was 3161.80, 3519.80, 2296.30, 13222, and 189.99 MPa·s, respectively. Prakash et al. [31] reported that a combination of guar gum and pectin had higher effects on the viscosity of jellies. Also, they reported that higher viscosity is a suitable property in the jelly formulations.

3.5. Rheological Properties. Jelly is one of the foods in the class of viscoelastic materials, and the rheological parameter of this food can be evaluated by a dynamic rheological method. The findings of frequency sweep analysis are shown in Figure 2. Three different types of systems, such as gel, concentrated solution, and dilute solution, can be studied by the frequency sweep test. For gel systems, storage moduli are higher than loss moduli over the applied frequency range. In dilute solutions, loss moduli dominate storage moduli which

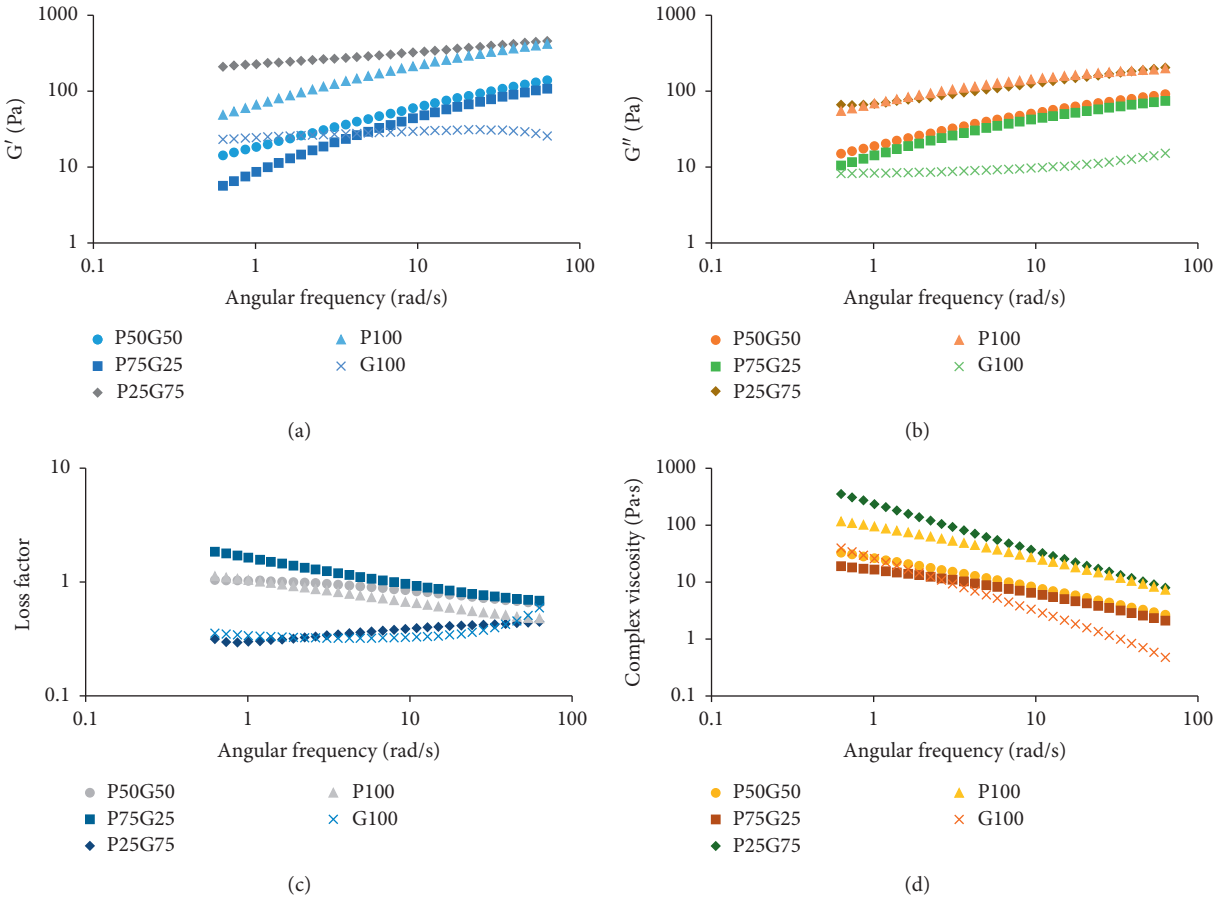

Figure 2: The effects of pectin: gum ratio on storage (G'), loss (G'') moduli, Tan δ, and complex viscosity of samples.

become close to each other at higher frequencies. In concentrated systems, storage moduli are lower than loss moduli at low frequency and crossover each other in the middle range of frequency [34]. The loss (G'') and storage (G') moduli of different samples showed relatively similar dependency on the applied frequency range. A decrease in G' was observed after replacement with gum indicating the low elastic character of gum. Also, the highest loss (G'') and storage (G') moduli was observed in the P25G75. The higher intermolecular interactions and entanglements between gum cordia chains and pectin could reinforce the biopolymeric network. The data of frequency sweep are in high agreement with those of apparent viscosity. In the P25G75 sample, apparent viscosity and frequency sweep parameters (G'', G', and complex viscosity) were highest.

Tan δ is the ratio of loss moduli (G'') to storage (G') moduli (Figure 2(c)). When tan δ is <1, samples have elastic behavior and when tan δ is >1, samples have viscous behavior. The system is not a real gel when tan δ is higher than 0.1 [35]. The results, reported in Figure 2(c), indicate the presence of weak gel structure (or dominant elastic behavior) in a wide range of applied frequency in samples containing 75 and 100% gum. The solid-like behavior of samples was similarly reported by Garrido et al. [36].

A decrease in complex viscosity ($\eta*$) of different samples was reported by increasing the angular frequency (Figure 2(d)), mainly due to the macromolecules connections and chains entanglements disruption. This behavior shows the shear-thinning behavior of apple jelly. Similar observations about decreasing complex viscosity were reported by Garrido et al. [36] and Figueroa and Genovese [21].

3.6. Textural Properties. Texture analysis can be divided into two instrumental and sensorial technique. Our study applied TPA in order to study the textural characterization of apple jelly [37]. The effects of pectin: gum ratio (0 : 100, 25 : 75, 50 : 50, 75 : 25 and 100 : 0) on textural properties (Hardness, Cohesiveness, Springiness, Gumminess, and Chewiness) of apple jelly were reported in Table 4. The results showed that with an increase in gum concentration, gel formation potential significantly decreased and in the sample containing 100% gum was liquid. The highest Hardness (2329.52 g), Gumminess (1298.60), and Chewiness (1212.59) were related to the sample containing 100% pectin. This phenomenon is due to the higher gel formation ability of pectin in comparison with gum. Similar results were reported by Slavutsky and Bertuzzi [38] and Ibáñez et al. [39]. The results showed that there are not any significant differences in Springiness. The highest Cohesiveness properties were related to the P75G25 sample.

Table 4: The effects of pectin: gum ratio on textural properties of samples.

	Hardness (g)	Cohesiveness	Springiness (g)	Gumminess	Chewiness
P50G50	751.79 ± 47.64C	0.58 ± 0.00B	0.88 ± 0.05A	433.67 ± 27.40C	384.20 ± 47.22C
P75G25	1796.24 ± 91.72B	0.62 ± 0.00A	0.88 ± 0.04A	1119.84 ± 52.51B	981.47 ± 86.32B
P25G75	207.21 ± 14.93D	0.45 ± 0.05B	0.95 ± 0.01A	92.74 ± 3.13D	87.78 ± 3.95D
P100	2329.52 ± 131.09A	0.56 ± 0.01B	0.93 ± 0.03A	1298.60 ± 98.38A	1212.59 ± 128.46A

*Capital letters in each column showed significantly differences ($P < 0.05$). *P50G50 (sample containing 50% pectin and 50% gum); P75G25 (sample containing 75% pectin and 25% gum); P25G75 (sample containing 25% pectin and 75% gum); P100 (sample containing 100% pectin) and G100 (sample containing 100% gum).

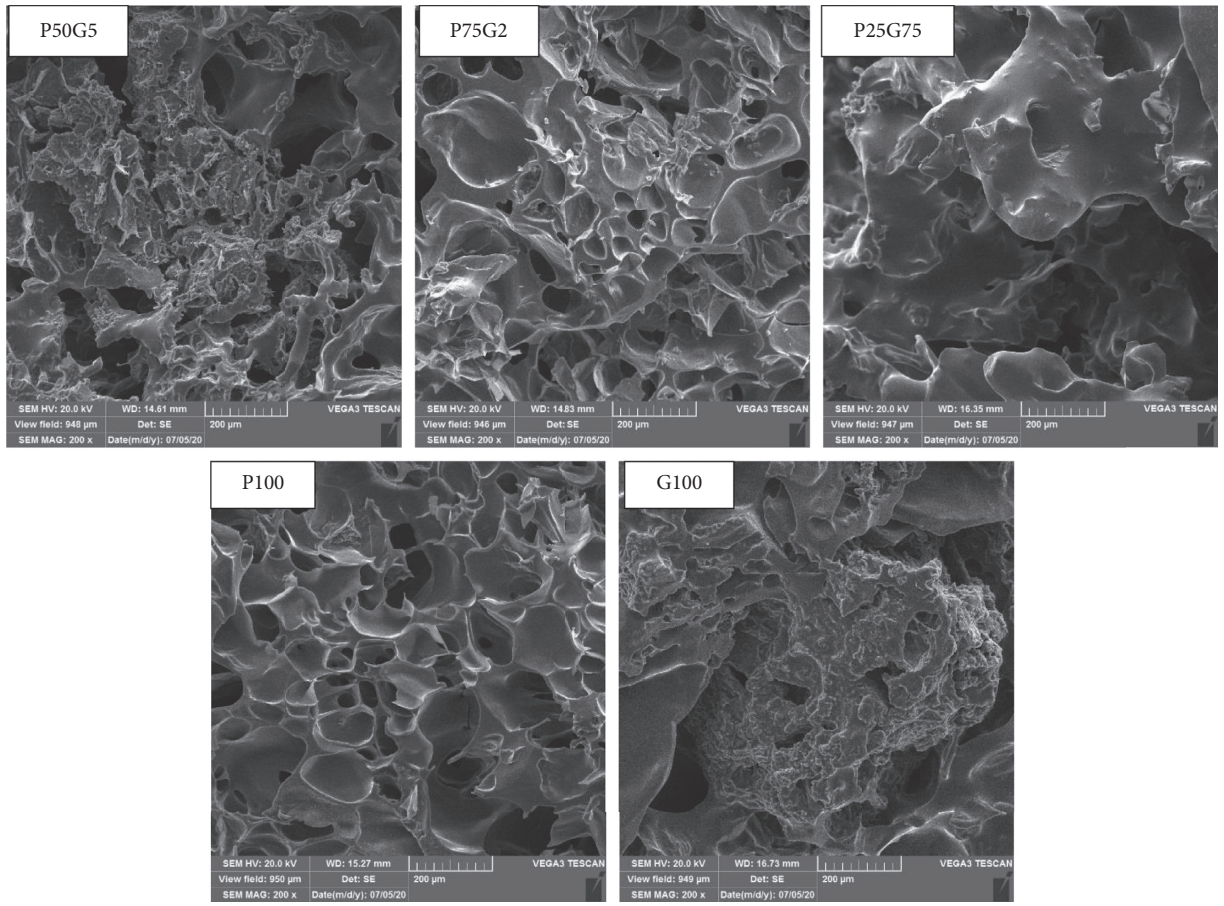

Figure 3: The effects of pectin: gum ratio on the microstructure of samples.

Table 5: The effects of pectin: gum ratio on the sensorial properties of samples.

	Odor	Texture	Taste	Appearance	Overall
P50G50	4.75 ± 0.25AB	3.75 ± 0.25AB	3.50 ± 0.50B	3.75 ± 0.25B	3.75 ± 0.25B
P75G25	5.00 ± 0.00B	3.75 ± 0.25AB	4.00 ± 0.41B	4.50 ± 0.29BC	4.50 ± 0.29C
P25G75	4.00 ± 0.41A	4.00 ± 0.00BC	3.50 ± 0.29B	4.50 ± 0.29C	4.25 ± 0.25C
P100	5.00 ± 0.00B	5.00 ± 0.00C	4.25 ± 0.48B	4.75 ± 0.25C	4.75 ± 0.25C
G100	4.00 ± 0.41A	3.00 ± 0.71A	2.25 ± 0.48A	2.25 ± 0.25A	2.75 ± 0.25A

*Data represent the means of three independent repeats ± standard deviation. *Capital letters in each column showed significant differences ($P < 0.05$). *P50G50 (sample containing 50% pectin and 50% gum); P75G25 (sample containing 75% pectin and 25% gum); P25G75 (sample containing 25% pectin and 75% gum); P100 (sample containing 100% pectin) and G100 (sample containing 100% gum).

3.7. Microstructure. The microstructure of the samples is shown in Figure 3. The sample containing 100 and 75% pectin had a uniform and regular structure, but with the addition of gum concentration, uniformity was decreased. Pectin samples present an open heterogeneous and tridimensional structure. This porous structure is obtained by the water molecules displacement during the drying process required by SEM observation [38]. Soares et al. [40] and

Oliveira Cardoso et al. [41] reported similar structures for hydrogels.

3.8. Sensorial Properties. Critical sensory attributes of the samples, including appearance, odor, texture, taste, and consumer acceptance, were determined by panelists [37]. Sensory properties samples are reported in Table 5. The results showed that sensory properties were significantly ($P > 0.05$) influenced by the gum cordia dose. The results showed sensorial properties of the sample containing 75% gum cordia was similar to the sample containing 100% pectin. This sample had a high score in texture, taste, appearance, and overall acceptability.

4. Conclusion

Today, consumers like healthier foods fortified with an antioxidant component such as phenolic ingredients. Our findings show that gum cordia can be applied as a stabilizer to production apple jelly. The total phenol content of the sample significantly increased with the addition of gums. Gum cordia had a significant influence on the improved viscoelastic parameters of the apple jellies. The rheological properties showed that the sample containing 75% gum cordia was a similar sample to control. Also, the sensorial properties showed that a sample containing 75% gum cordia had high score in texture, taste, appearance, and overall acceptability. The results suggested that gum cordia as a polymer can be successfully employed for the formulation of jelly for improving the technofunctional properties of jelly.

Data Availability

All data and analyses were reported as figures and tables in the manuscript.

Conflicts of Interest

The authors declare that they do not have any conflicts of interest.

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