Natural Preservatives to Improve Food Quality and Safety

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Guest Editors: Simona de Niederhausern, Chrissanthy Papadopoulou, Andrea Laukova, and Patrizia Messi
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Editorial

Natural Preservatives to Improve Food Quality and Safety

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Received 12 October 2017; Accepted 12 October 2017; Published 12 December 2017

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Food products can be contaminated by a variety of pathogenic and spoilage microbiota, the former causing foodborne diseases and the latter causing significant economic losses for the food industry due to undesirable effects on the food properties. According to the Centers for Disease Control and Prevention (CDC), each year in the United States, 48 million people get sick, 128,000 are hospitalized, and 3,000 die because of foodborne infections. However, although foodborne outbreaks are well recorded, sporadic cases are not because not all patients visit the doctor or enter the hospital and the causative agent is not always identified. A considerable number of foodborne pathogens of great public health importance (e.g., VTEC E. coli serotypes, Campylobacter jejuni, Listeria monocytogenes, and Yersinia enterocolitica) have emerged during the recent two decades causing severe illness and foodborne outbreaks worldwide.

Microbial food spoilage is also an area of immense concern for the food industry. It is estimated that as much as 25% of all food produced is lost after harvest due to microbial activity. While the growth of spoilage microbiota in foods is not harmful for the human health, it has negative impact on the shelf-life, textural characteristics, and overall quality of the finished products, affects the consumer choices, and results in significant commercial losses. Thus, prevention or inhibition of microbial growth in foods is of utmost importance for the current globalized food production. Hence, there is still the need for new processing methods, to be used either alone or in combination with the already existing ones, able to reduce or eliminate foodborne pathogens and spoilage bacteria.

Chemical additives have been extensively used to prevent the survival and proliferation of microorganisms, but their safety and impact on human health are under discussion. Since the reduction or elimination of pathogens and spoilage microorganisms in food is the foremost priority, the current trends in food processing are focusing on the use of natural compounds, which are considered as safe alternatives and satisfy the consumer preferences for more “green foods.” Hence the increased awareness on the safety of food additives and preservatives, and the consumer’s trend to avoid foods containing chemicals, which, in the long-term, may have adverse impact on their health, have generated a significant number of studies and publications on the potential use of various natural substances, recognized as GRAS (Generally Recognized as Safe), to be used as food preservatives. Chemical compounds added to foods as antioxidant agents are also of concern, particularly when important protein sources (e.g., burgers, steaks) are involved in the daily diet of westernized consumer living in the developed countries [1, 2]. Synthetic antioxidants such as BHT, TBHQ, and BHA have demonstrated various adverse effects on the human health including allergy, headache, asthma, and dermatitis. Recent studies on the utilization of natural antioxidants (e.g., herbal essential oils and extracts) indicate their capacity and safety [3, 4]. Also, the antimicrobial properties of natural substances such as plant essential oils and extracts have been extensively studied with promising results [5]. The proper recycling and use of fruit-processing plants by-products like fruit seeds or skins discarded and piling up in huge amounts every year, are of great interest for the food industry, particularly their potential to be a useful source of oil and meal [6]. Additionally, the oil from plant seeds can be used by the food industry for manufacturing more “natural” or “green” foods and also can sufficiently extend the shelf-life of the...
food product [7]. The oils derived from fruit seeds oils may also have some more useful properties beyond being just “edible.” The oil derived from the seed extract of two Mexican varieties of cactus pear (Opuntia albicarpa and Opuntia ficus indica) has been found to have sufficient antioxidant and antimicrobial properties [8]. Furthermore, some biological active compounds produced by microorganisms are widely used in the food industry as well. A good alternative to conventional chemically synthesized food preservatives is the use of natural antimicrobials such as bacteriocins produced by lactic acid bacteria (LAB), with Nisin being officially employed by the food industry and thus the most widely used. Bacteriocins are peptides, actually microbial toxins, produced from various microorganisms, and so far some of them (e.g., enterocins produced mostly by enterococci) have shown remarkable antimicrobial potential and their application as a natural barrier against pathogens and food spoilage has been proven to be very efficient, when used in the form of purified or semipurified extracts or as protective cultures [9, 10]. Therefore, as the developments in food preservation are focusing on the implementation of natural antimicrobials and antioxidants this special issue explores the potential of alternatives to currently used preservatives through the publication of five high-quality articles, which aim to address recent advancements in the field of natural food preservatives and antioxidants. The prospect to replace synthetic preservatives with natural substances has been demonstrated by comparing the effects of Shirazi thyme, cinnamon, and rosemary extracts (denoted as natural antimicrobials) with those of the synthetic antioxidant BHT on protein and lipid oxidations, physicochemical, microbial, and sensory characteristics of frozen beef burgers during storage. Specifically, the oxidative stability of the beef burgers containing Shirazi thyme, cinnamon, and rosemary extracts was well demonstrated, and the antibacterial activity was documented, as the total microbial counts of the tested burgers were well below the maximum allowed limit. Besides the evaluation of the antimicrobial and antioxidant properties of natural substances it is important to optimize their recovery. With regard to bacteriocins, to enhance Enterocin Y31 production and simplify the steps of separation and purification, a proper simplified and defined medium (SDM) has been developed for the Enterococcus faecium Y31 growth and enhancement of the Enterocin Y31 production. The bacterial growth did not result in Enterocin Y31 production in MRS medium and, therefore, both the growth rate and the Enterocin Y31 production were set as the goal for the investigation. Single omission experiments revealed that 5 g/L NaCl, five vitamins, two nucleic acid bases, MgSO4⋅7H2O, MnSO4⋅4H2O, KH2PO4, K2HPO4, CH3COONa, fourteen amino acids, and glucose were essential for the adequate strain growth and Enterocin Y31 production. As a result, a novel simplified and defined medium (SDM) was formulated containing 30 ingredients, in which the Enterocin Y31 production yield was higher when compared to either MRS or CDM. The development of the SDM improved the Enterocin Y31 production and simplified the steps of purification (only two steps), which is very promising and increases its potential applications. Referring to seeds’ oils, the optimization of the extraction conditions of cactus pear seed oil has been obtained using ultrasound in a closed system based on the antioxidant activity and using response surface methodology. The yield extraction and antioxidant and antimicrobial activity were compared with those obtained using both conventional and unconventional methods, such as Soxhlet and maceration, using heat, agitation or long extraction times, microwave, supercritical fluids and ultrasound-assisted extraction in an open and in a closed system. The results have shown that ultrasound exhibited lower oil yield and antioxidant activity but had the potential to achieve comparable results if multiple ultrasound extractions are performed in the time needed by conventional methods. Seed oils showed similar antimicrobial activity despite the extraction method and can be an alternative extraction method of seed oils from fruits such as cactus pear. Lastly, the replacement of chemical additives with natural compounds has been addressed for fish foods as well. Fish-paste products also known as fish cakes or surimi-based products are worldwide favorites. Surimi, a wet protein concentrate of fish muscle, is used as an intermediate raw material to produce surimi seafood. The flavor, texture, taste, shelf-life, and market value of surimi-based products depend on several factors, including the additives used to prepare the surimi. While preparing surimi with chemical additives, several problems have been observed, such as a lack of unique characteristics, inferior acceptability, and poor functionality. In this context, a systematic review of fish-paste products prepared using natural food additives (e.g., animal, seafood and plant source additives, herbs and oriental medicines, grains and roots, and functional food materials) has been performed, which summarizes the existing relevant knowledge in the production of new value-added foodstuffs of interest for the surimi industry.

In conclusion, this special issue offers to the readers the chance to be informed on the recent advancements related to the antimicrobial and antioxidant properties of natural substances that are of interest for the contemporary food industry. Their prospective use in the food production has the potential to lead towards the production of safer and healthier foods, not excluding their contribution to a more efficient preservation of the environment, when chemicals will be replaced with natural substances.

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Review Article

Natural Food Additives and Preservatives for Fish-Paste Products: A Review of the Past, Present, and Future States of Research

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Received 9 June 2017; Revised 15 August 2017; Accepted 17 September 2017; Published 27 November 2017

Academic Editor: Moreno Bondi

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Fish-paste products, also known as fish cakes or surimi-based products, are worldwide favorites. Surimi, a wet protein concentrate of fish muscle, is used as an intermediate raw material to produce surimi seafood. The flavor, texture, taste, shelf-life, and market value of surimi-based products depend on the source of the fish meat, type of applied heat treatment, and additives used to prepare the surimi. While preparing surimi with chemical additives, several problems have been observed, such as a lack of unique characteristics, inferior acceptability, and poor functionality. Various types of fish-paste products have been developed by using different ingredients (e.g., vegetables, seafood, herbs and oriental medicines, grains and roots including carrots, and functional food materials). However, a systematic review of fish-paste products prepared using natural food additives has not yet been performed. Therefore, the quality characteristics of fish-paste products and their functionalities were elucidated in this study. With the increasing demand for surimi seafood products, the functional properties, physiochemical properties, and shelf-life of surimi-based products need to be improved. This review will aid the preparation of new value-added products in the surimi industry.

1. Introduction

Fish-paste products, popularly known as fish cakes, are produced from frozen surimi (i.e., they are a kind of surimi-based product) and are popular in Korea and Japan [1]. In the Korean Food Standards Codex, fish cakes are known as a processed marine product comprising salt-soluble proteins isolated from fish meat [2]. Fish muscle is mechanically deboned, washed with water, and blended with cryoprotectants to prepare a wet concentrate of proteins called surimi. Surimi is a Japanese term that is also known as washed fish mince. It is a refined fish myofibrillar protein manufactured through numerous step-by-step processes including heading, gutting, filleting, deboning (mincing), washing, dewatering, refining, mixing with cryoprotectants, freezing, and metal detection for HACCP [3]. The myofibrillar proteins make it an excellent ingredient for developing food products. It has excellent gelling properties and forms strong and elastic gels upon heating [4].

The setting and deformation are important to prepare surimi and surimi-based products. Setting, also known as “suwari” in Japanese, is a very important process, which has a significant influence on the physiological and rheological properties of surimi-based products. Setting is a vital process in the quality estimation of surimi because it helps to improve the water-holding capacity as well as the gel texture of surimi-based products. When fish mince paste (sol) is heated at a low temperature (up to 50°C), a loose network (suwari) is formed.
from myosin and actomyosin molecules; this process is referred to as setting. Setting is species dependent and occurs over a range of temperatures (up to 50°C) and to a varying extent. As the temperature is increased to around 70°C, suwari is partially disrupted to form a broken net structure (modori), a phenomenon attributed to the dissociation of myosin from actin and the possible fragmentation of the actin filament [5–7].

Most of the surimi-based products are different types of fish-paste products, while less than 10% include fish burgers, fish ham, and fish sausages [3]. Surimi-based products are prepared by mixing the raw or frozen surimi with salts and other ingredients, molded and heated to form the final product in the specified shape and texture, and pasteurized before packaging. The kind of heat treatment depends on the flavor, texture, and appearance of the desired final product and may include broiling, steaming, deep-fat frying, and boiling [8], while fish-paste products in South Korea are mostly prepared by frying [9]. Textural characteristics such as gel strength are the major determinant of surimi price and quality [10]. Several studies have attempted to enhance the textural quality of surimi or surimi-based products using various protein additives [11–14].

Surimi quality and gelling property are mainly affected by both intrinsic factors (effect of fish species, seasonality, sexual maturity, and freshness or rigor) and extrinsic factors (harvesting, handling, water characteristics, processing time and temperature, solubilization of myofibrillar proteins during processing, the activity of the endogenous or added protein oxidants, and proteolytic enzymes, washing cycles, salinity, and pH) [3, 134]. Surimi forms thermoirreversible gels upon heating, which do not deform with further change in the temperature. This phenomenon of surimi and surimi-based products is similar to that observed in other proteins, such as egg white, milk-lactoglobulin, and wheat gluten. Additionally, surimi produces gels of very high deformability and strength. This heat-induced gelation property of surimi makes it a very valuable food ingredient [134].

The gel-forming ability and capacity of surimi are adversely affected by the proteolytic degradation of myofibrillar proteins. The presence of indigenous proteinases caused gel softening in surimi made from fish species, for example, threadfin bream [135], arrow tooth flounder [136], Pacific whiting [137], and lizard fish and bigeye snapper [20, 59]. Various active proteinases in fish muscle are responsible for softening of the surimi gels. Nakamura and Ogawa and An et al. testified that cathepsins L and B were the most active cysteine proteinases in Pacific whiting surimi and fish fillets, respectively [7, 138]. The myofibrill-associated proteinases observed in lizardfish surimi were serine proteinases and cysteine, while a serine proteinase was reported in the bigeye snapper surimi [20, 59].

Seasonal analysis of the compositional properties of Alaska pollock and Pacific whiting showed higher protein contents in winter, while the moisture contents were higher in summer [139, 140]. Surimi prepared from cold-water fish species with low thermostability of myofibrillar proteins makes setting easier. Normally, a myofibrillar protein with low thermostability is optimum to do setting because its reactivity is increased due to the loose internal structure by sodium chloride addition and heat denaturation. In contrast, the myofibrillar protein of tropical fish species with high heat stability is difficult to denature and form the myofibrillar protein network structure in surimi [3].

Several research groups have studied ways to enhance the quality of surimi-based products by investigating the changes in microbial content, enzyme activity, nutrient content and acceptability characteristics, the use of raw materials, the standardization of the manufacturing process, storage, and marketing. Natural and chemical food-grade additives such as konjac flour, proteinase inhibitors, egg white, and hydrocolloids have been used to enhance the gelling properties of surimi [12, 17, 20, 23, 34, 141–143]. The trypsin inhibitor in egg white plays a major role in improving the gel strength of surimi. The addition of egg white inhibits the proteolytic activity of the modori-inducing enzyme in fish meat and suppresses the decrease of elasticity in surimi. Serum proteinases have strong inhibiting abilities against the action of various proteases with different active centers such as SH groups and serine groups. In addition, they also contain transglutaminases that accelerate the setting. Egg white and serum proteins also play an important role in inhibiting the enzymatic activity of the parasites. Apart from the use of additives, gel strengthening can be achieved by treating the gels at low temperatures (0–40°C) before cooking [144, 145]. Furthermore, the gel quality of surimi and surimi-based products can also be enhanced by using alternate fish species, or by acid and alkali washing to increase the myofibrillar protein concentrations [7, 146, 147].

Surimi production worldwide reached around 800,000 MT by 2011–2012 [3], while South Korea alone contributed approx. 156,000 MT in 2013. Over the years, the size of the market for Korean fish-paste products has gradually increased, reaching ~350 million US dollars in 2013 (based on the amount produced) [148]. However, manufacturers have had several problems in producing surimi-based products, such as a lack of unique characteristics and inferior acceptability and functionality. Therefore, there have been several attempts to develop new fish-paste products with excellent acceptability and functionality. To this end, numerous types of fish-paste products have been developed using various natural ingredients such as vegetables, seafood, animals, plants, herbs and oriental medicines, seaweed, and functional food materials. It is of worldwide interest to use these natural food preservatives instead of chemical or synthetic ones. However, to date, no systematic review of fish-paste products supplemented with various food raw materials has been performed. Therefore, the quality characteristics of fish-paste products and their functionalities were elucidated in this study.

2. Improvement of the Gel Properties of Fish-Paste Products

Various food additives derived from animals (e.g., beef, swine, and chicken), seafood (e.g., fish, invertebrates), plants (e.g., legumes, cereals), sugars, polyols, and functional materials used in fish-paste products to improve their gelling
The autolytic activities of lizardfish increase the breaking strength and gel deformation [28]. The addition of 2% ovomucoid could inhibit [24, 25]. Plasma at a level of 2% inhibited the degradation of myosin and arrowtooth flounder mince were reported by Rawdkuen et al. [22]. Furthermore, similar results were observed in the extended study on Pacific whiting surimi, where chicken inhibitor fraction from chicken plasma on Pacific whiting protein was supplemented to sardine kamaboko gels at levels [19]. The effect of porcine plasma protein on the bigeye snapper (Priacanthus tayenus) surimi gel characteristics was investigated by Benjakul et al. [14]. The gels supplemented with 0.5% porcine plasma protein had the highest level of deformation and breaking force. Benjakul and colleagues later reported the effect of porcine plasma protein on the gel characteristics of surimi from bigeye croaker (Penna harmed at a concentration of 2%, with a yellow hue color [16]. Weerasinghe et al. characterized the inhibitory activity of these food-grade inhibitors and reported that the inhibitory activity was mainly because of specific serine proteinase inhibitors [17]. The addition of 2% dried bovine plasma to steamed fish-paste slightly increased the chewiness and hardness, but it showed a negative effect on the gel strength [18]. Duangmal and Taluengphol reported that the higher levels of beef plasma protein unfavorably affected gel characteristics of red tilapia surimi gels [19].

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Ovomucoid is a mucoprotein obtained from egg white that has been tested for its potential as a gel-degradation inhibitor [24, 25]. The addition of 2% ovomucoid could increase the breaking strength and gel deformation [28]. The autolytic activities of lizardfish (Saurida tumbil) surimi and mince under the application of protease inhibitors were investigated by Yongsawatdigul and Piya phamnaviboon [26]. At all preincubation conditions, egg white enhanced the gel-forming capability of S. tumbil surimi to a greater extent than did whey protein concentrate. The addition of 1% egg white and preincubation at 25°C increased the breaking force by twofold. Campo-Deaño and Tovar [27] reported that the addition of egg albumen at 1.5% and 2% for Alaska pollock and Pacific whiting surimi, respectively, enhanced the gel strength of crab sticks. According to Hunt et al., the incorporation of 2%-3% special dried egg white improved the gel textural characteristics of Alaska pollock and Pacific whiting surimi [28].

Whey is the complete set of proteins isolated from the watery portion of milk. Whey protein is comprised of 20% milk protein and 80% casein [149]. Whey protein concentrates have generally been used as an emulsifier, filler, water binder, protein supplement, foam stabilizer, and thickener as well as gelling agent [150]. Rawdkuen and Benjakul investigated the effect of whey protein concentrate on the gelling characteristics of surimi prepared from goatfish (Mullloidichthys vanicolensis), bigeye snapper (P. tayenus), lizardfish (S. tumbil), and threadfin bream (N. bleekeri) [29]. All the tested surimi supplemented with 3% whey protein concentrate displayed inhibitory activity against autolysis and significantly reduced gel whiteness. However, better water-holding capacity was obtained by increasing concentrations of whey protein concentrate.

Plasma proteins produced from pig, cow, and chicken byproducts are relatively affordable and easily collectible sources [143, 151, 152]. However, outbreaks of foot-and-mouth disease, avian influenza, and mad cow disease, as well as a ban on proteins from pig bone and skin in some states for religious causes, have made it essential to search alternative sources [153, 154]. Various fish plasma proteins have been tested, including those from rainbow trout and salmon [31, 35, 36].

2.2. Seafood Additives. The effects of shrimp head protein hydrolysate from different shrimp, namely, black tiger shrimp (Penaeus monodon), northern pink shrimp (Pandalus eous), and endeavour shrimp (Metapenaeus endeavouri) on the gelling properties of lizardfish (Saurida spp.) surimi were investigated by Ruttanapornvareesakul et al. [30]. It was reported that the freeze-induced denaturation of lizardfish muscle protein could be reduced by the supplementation of shrimp head protein hydrolysate at a concentration of 5%, resulting in higher Ca-ATPase activity and gel strength. The effects of rainbow trout plasma proteins on the gelling properties of surimi prepared by Alaska pollock were investigated by Li et al. [31]. Gel degradation, deformation, the breaking force, water-holding capacity, and whiteness enhanced with increasing amounts of rainbow trout plasma protein and decreased at higher concentrations. The rainbow trout plasma protein at a concentration of 0.75 mg/g could be used as a potential protease inhibitor to inhibit gel weakening in Alaska pollock surimi. Li et al. reported the higher inhibitory activities of the recombinant chum salmon cystatin against autolysis of Alaska pollock surimi [155].

Fish gelatin is extracted from the collagen of fish skin and it is used as a food additive. Hernández-Briones et al. studied the functional and mechanical properties of Alaska pollock surimi gels while using fish gelatin as an additive [32]. The increasing concentration of gelatin affected the whiteness but the sensory panelists were unable to detect it. These results showed that fish gelatin was not effective as a functional additive in Alaska pollock surimi. Nevertheless, it could be added at up to 10 g/kg without negatively affecting the mechanical properties of surimi. Yin et al. reported a significant improvement in the endogenous transglutaminase activity of Alaska pollock surimi prepared with nanoscaled.
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<td>Atlantic menhaden (<em>Brevortia tyrannus</em>), Alaska pollock (<em>Theragra chalcogramma</em>), Arrowtooth flounder (<em>Atheresthes stomias</em>), Walleye pollock</td>
<td>Moisture content, protein content, cooking loss, water-holding capacity, texture, torsion test</td>
<td>0.5%–1.5%</td>
<td>[15]</td>
</tr>
<tr>
<td>Bovine plasma</td>
<td><em>Bos taurus</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Pacific whiting (<em>Merluccius productus</em>)</td>
<td>Protein content, inhibitory assay</td>
<td>2%</td>
<td>[16]</td>
</tr>
<tr>
<td>Beef plasma protein</td>
<td><em>Bos taurus</em></td>
<td>—</td>
<td>Powder</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Nutrient content, pH, water-holding capacity, texture and sensory evaluation</td>
<td>2%</td>
<td>[17]</td>
</tr>
<tr>
<td>Dried bovine plasma</td>
<td><em>Bos taurus</em></td>
<td>Steaming</td>
<td>Powder</td>
<td>Red tilapia (<em>O. niloticus</em> x <em>O. placidus</em>), Bigeye snapper (<em>Priacanthus tayenus</em>), Threadfin bream (<em>Nemipterus bleekeeri</em>), Bigeye croaker (<em>P. tayenus</em>), Bigeye croaker (<em>Pennahia macrophthalmus</em>)</td>
<td>Color, texture, expressive water, protein, total sulfhydryl content</td>
<td>2 g/kg</td>
<td>[18]</td>
</tr>
<tr>
<td>Beef plasma protein</td>
<td><em>Sus scrofa domesticus</em></td>
<td>Heated in a water bath</td>
<td>Dried powder</td>
<td>Baracuda (<em>Sphyraena jello</em>) and Bigeye croaker (<em>Pennahia macrophthalmus</em>)</td>
<td>Trichloroacetic acid-soluble peptides, color, texture, protein content</td>
<td>0.5%</td>
<td>[19]</td>
</tr>
<tr>
<td>Porcine plasma protein</td>
<td><em>Sus scrofa domesticus</em></td>
<td>Heated in a water bath</td>
<td>Dried powder</td>
<td>Sardine (<em>Sardinella gibbosa</em>)</td>
<td>Color, texture, expressive moisture, protein content, autolysis activity</td>
<td>2%</td>
<td>[20]</td>
</tr>
<tr>
<td>Cysteine proteinase inhibitor from Chicken plasma</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Dried powder</td>
<td>Arrotooth flounder (<em>A. stomias</em>), Pacific whiting (<em>M. productus</em>)</td>
<td>Autolysis and inhibitory activity, pH, protein content</td>
<td>3%</td>
<td>[21]</td>
</tr>
<tr>
<td>Chicken plasma</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Dried powder</td>
<td>Pacific whiting (<em>M. productus</em>)</td>
<td>Torsion and fracture test, dynamic rheological attribute</td>
<td>2%</td>
<td>[22]</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Ovomucoid solution</td>
<td>Alaska pollock</td>
<td>Puncture test, textural and sensory attributes</td>
<td>2%</td>
<td>[23]</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Ovomucoid solution</td>
<td>Alaska pollock</td>
<td>Puncture test, textural and sensory attributes</td>
<td>2%</td>
<td>[24]</td>
</tr>
<tr>
<td>Egg white (EW)</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Lizardfish (<em>Saurida tumbil</em>), mince and surimi Surimi-based crab sticks from Alaska pollock (AP), Pacific whiting (PW); (<em>M. productus</em>)</td>
<td>Transient test, strength test, texture, dynamic rheological and physical attributes</td>
<td>AP: 1.5%, PW: 2%</td>
<td>[25]</td>
</tr>
<tr>
<td>Egg albumin</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in the cooking roller</td>
<td>Egg white itself</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common name</td>
<td>Species</td>
<td>Cooking method</td>
<td>Used as</td>
<td>Fish source for surimi</td>
<td>Metrics</td>
<td>Optimum amount or treatment condition</td>
<td>References</td>
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<tr>
<td>Regular dried egg white (REW), special dried egg white (SEW), liquid egg white (LEW)</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Spray-dried powder</td>
<td>Pacific whiting (<em>M. productus</em>)</td>
<td>Total sulphydryl groups, fracture test, dynamic rheological attribute</td>
<td>SEW: 2%-3%</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bigeye snapper (<em>P. tayenus</em>), Goatfish (<em>Malloidyichthys vanicolensis</em>), Threadfin bream (<em>N. bleekeri</em>), and Lizardfish (<em>S. tumbil</em>)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Whey protein concentrate</td>
<td><em>Bos taurus</em></td>
<td>Heated in a water bath</td>
<td>Whey protein concentrate</td>
<td></td>
<td>Water-holding capacity, color, autolytic activity</td>
<td>3%</td>
<td>[29]</td>
</tr>
<tr>
<td>Seafood additives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrimp head protein hydrolysate from northern pink shrimp, endeavour shrimp, and black tiger shrimp</td>
<td><em>Pandalus eous, Metapenaeus endeavouri, Penaeus monodon</em></td>
<td>Heated in a water bath</td>
<td>Dried matter</td>
<td>Lizardfish (<em>Saurida spp.</em>)</td>
<td>Gel strength, color, gel-forming ability, Ca-ATPase activity</td>
<td>5%</td>
<td>[30]</td>
</tr>
<tr>
<td>Rainbow trout plasma protein</td>
<td><em>Oncorhynchus mykiss</em></td>
<td>Heated in a water bath</td>
<td>Freeze-dried plasma</td>
<td>Alaska pollock</td>
<td>Proximate analysis, water-holding capacity, color, texture, protein content</td>
<td>0.75 mg/g</td>
<td>[31]</td>
</tr>
<tr>
<td>Fish gelatin</td>
<td>Commercial fish gelatin (<em>Gelatin Rousselot</em>)</td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Alaska pollock</td>
<td>Color, mechanical, functional, sensory attributes</td>
<td>10 g/kg</td>
<td>[32]</td>
</tr>
<tr>
<td>Nanoscaled fish-bone of Pacific whiting</td>
<td><em>M. productus</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Alaska pollock</td>
<td>Texture, scanning electron microscopy</td>
<td>1 g/100 g</td>
<td>[33]</td>
</tr>
<tr>
<td>Nanoscaled fish-bone (NFB) + dried egg white (DEW)</td>
<td><em>M. productus, Gallus gallus domesticus</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Pacific whiting (<em>M. productus</em>)</td>
<td>Rheological and textural attributes</td>
<td>DEW: 1% + 10 mg NFB Ca/g surimi paste</td>
<td>[34]</td>
</tr>
<tr>
<td>Salmon blood plasma</td>
<td><em>Oncorhynchus tshawytscha</em></td>
<td>Ohmic heating</td>
<td>Freeze-dried plasma</td>
<td>Pacific whiting (<em>M. productus</em>)</td>
<td>Scanning electron microscopy, protein content, dynamic rheological attributes</td>
<td>1 g/100 g</td>
<td>[35]</td>
</tr>
<tr>
<td>Common name</td>
<td>Species</td>
<td>Cooking method</td>
<td>Used as</td>
<td>Fish source for surimi</td>
<td>Metrics</td>
<td>Optimum amount or treatment condition</td>
<td>References</td>
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<tr>
<td>Freeze-dried chinook salmon plasma (FSP) and concentrate salmon plasma (CSP)</td>
<td>Oncorhynchus tshawytscha</td>
<td>Ohmic heating</td>
<td>Freeze-dried plasma</td>
<td>Pacific whiting (M. productus) surimi and Salmon mince</td>
<td>Proteolytic inhibition, autolysis, protein content</td>
<td>Salmon mince: CSP &gt; FSP</td>
<td>Pacific whiting surimi: FSP [36]</td>
</tr>
<tr>
<td>Partially purified trypsin inhibitor from the roe of yellowfin tuna fish Squid ink tyrosinase (SIT) + tannic acid (TA)</td>
<td>Thunnus albacares</td>
<td>Heated in a water bath</td>
<td>Freeze-dried</td>
<td>Bigeye snapper (Priacanthus macracanthus)</td>
<td>Proteolysis, color, water-holding capacity, gelling properties</td>
<td>3 g/100 g</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>Todarodes pacificus</td>
<td>Heated in a water bath</td>
<td>Mixture</td>
<td>Sardine (Sardinella albella)</td>
<td>Tyrosinase activity, in vitro oxidation assay, color, textural and sensory attributes</td>
<td>SIT: 500 U/g protein + TA: 1%</td>
<td>[38]</td>
</tr>
</tbody>
</table>

<p>| Plant source additives | | | | | | | |
| Soybean protein, wheat gluten | Glycine max, Triticum aestivum | Heated in a water bath | Soybean protein, Wheat gluten | Alaska pollock (T. chalcogramma) | Expressible water, moisture content, gel strength, physical attributes | 5% | [39] |
| Soy protein, egg white (EW), whey protein concentrate (WPC), Lactalbumin (LA), milk protein isolate (MPI) | Glycine max, Gallus gallus domesticus, Bos taurus | Cooked in a steam cooker | Powder | Alaska Pollock (T. chalcogramma) | Texture, expressible moisture content, water retention properties | EW and MPI | [40, 41] |
| Legume seed extract from, black cowpea, white cowpea, soybean seeds, Mungbean, peanut | Vigna unguiculata, Glycine max, Vigna radiata, Arachis hypogaea | — | Freeze-dried proteinase inhibitor extracts | Threadfin bream (Nemipteridae) | Thermal stability, pH, protein content, proteinase inhibitory assay | Black cowpea, soybean seeds: 30 mg/g | [42] |
| Legume seed extract from Cowpea, pigeon pea, bambara groundnuts bambara groundnut protein isolate | Vigna unguiculata, Cajanus cajan, Voandzeia subterranea | — | Partially purified Trypsin | Threadfin bream (Nemipteridae) | Sarcoplasmic modori-inducing proteinase activity, color | 30k units/g | [43] |
| Soy protein isolate | Vigna subterranea | Heated in a water bath | Powder | Threadfin bream (N. bleekeri) | Color, autolysis | 0.25 g/100 g | [44] |
| | Glycine max | Heated in a water bath | Commercial soy protein isolate (jinQui 1200) | Alaska pollock (T. chalcogramma), Common carp (Cyprinus carpio) | Total nitrogen and moisture content, gel strength, color | 10% | [45] |</p>
<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Cooking method</th>
<th>Used as</th>
<th>Fish source for surimi</th>
<th>Metrics</th>
<th>Optimum amount or treatment condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean protein, wheat gluten</td>
<td><em>Glycine max</em>, <em>Triticum aestivum</em></td>
<td>Heated in a water bath</td>
<td>Soy protein, wheat gluten</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Expressible water, moisture content, gel strength, physical attributes</td>
<td>5%</td>
<td>[46]</td>
</tr>
<tr>
<td>Dietary fiber (DF) from pea and chicory + microbial transglutaminase (MTGase)</td>
<td><em>Cichorium intybus</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>—</td>
<td>—</td>
<td>MTGase: 100 U/g</td>
<td>[47]</td>
</tr>
<tr>
<td>Protein isolates from Mengbean (MBPI), black bean (BBPI), bambara groundnut (BGPI)</td>
<td><em>Phaseolus aureus</em>, <em>Phaseolus vulgaris</em>, <em>Vigna subterranea</em></td>
<td>—</td>
<td>Freeze-dried powder</td>
<td>—</td>
<td>—</td>
<td>1 g/100 g</td>
<td>[48]</td>
</tr>
<tr>
<td>Partially purified trypsin inhibitor from adzuki bean</td>
<td><em>Vigna angularis</em></td>
<td>Heated in a water bath</td>
<td>Freeze-dried powder</td>
<td>Threadfin bream (<em>N. bleekerii</em>)</td>
<td>Protein content, texture, color, trypsin inhibitory activity assay, autolytic activity assay</td>
<td>3 g/100 g</td>
<td>[49]</td>
</tr>
<tr>
<td>Amylose (A) and amylopectin (AP)</td>
<td><em>Triticum aestivum</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Walleye pollock (<em>T. chalcogramma</em>)</td>
<td>Gelation and breaking strength</td>
<td>Amylose: 70% + Amylopectin: 4%</td>
<td>[50]</td>
</tr>
<tr>
<td>Wheat starch</td>
<td><em>Solanum tuberosum</em></td>
<td>Heated in the Krehalone casing film</td>
<td>Powder</td>
<td>Pacific sand lance (<em>Ammodytes personatus</em> Girard)</td>
<td>Proximate analysis, protein composition, color, folding text, textural and sensory attributes</td>
<td>8%</td>
<td>[53]</td>
</tr>
<tr>
<td>Potato starch and Modified sweet potato starch (NSPS) and Modified sweet potato starch (MSPS)</td>
<td><em>Ipomoea batatas</em></td>
<td>Heated on a controlled stress rheometer</td>
<td>Powder</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Dynamic rheological attributes</td>
<td>5%</td>
<td>[52]</td>
</tr>
<tr>
<td>Cryoprotectants and humectants</td>
<td><em>Ceratonia siliqua</em></td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Silver carp (<em>Hypophthalmichthys molitrix</em>)</td>
<td>Torsion test, gel-forming ability, mechanical attributes</td>
<td>X/LB: 0.25/0.75</td>
<td>[57]</td>
</tr>
<tr>
<td>Xanthan (X), locust bean (LB) gums alone, X/LB ratio</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Gum and powder</td>
<td>Silver carp (<em>H. molitrix</em>)</td>
<td>Water-holding capacity, mechanical and textural attributes</td>
<td>Pectin gum: 1% + CaCl₂: 0.2%</td>
<td>[58]</td>
</tr>
<tr>
<td>Common name</td>
<td>Species</td>
<td>Cooking method</td>
<td>Used as</td>
<td>Fish source for surimi</td>
<td>Metrics</td>
<td>Optimum amount or treatment condition</td>
<td>References</td>
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</tr>
<tr>
<td>Chitosan 7B from prawn shell</td>
<td>Not mentioned</td>
<td>Heated in a water bath</td>
<td>Not mentioned</td>
<td>Barred garfish (<em>Hemiramphus far</em>)</td>
<td>Protein content, SEM, textural attributes</td>
<td>1%</td>
<td>[59]</td>
</tr>
<tr>
<td>Konjac glucomannan aqueous dispersion</td>
<td><em>Amorphophallus konjac</em></td>
<td>Heated in a water bath</td>
<td>Aqueous dispersion</td>
<td>Giant squid (<em>Dosidicus gigas</em>), Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Protein solubility, pH, textural and viscoelastic rheological attributes</td>
<td>1%</td>
<td>[60]</td>
</tr>
<tr>
<td>Carrageenan + NaCl or KCl</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Hydrocolloid</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Gel strength, color, compression test</td>
<td>Carrageenan: 1% + KCl: 1.5%</td>
<td>[61]</td>
</tr>
<tr>
<td><em>Amorphophallus konjac</em> flour (AKF) NaCl + high hydrostatic pressure (HHP)</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Flour</td>
<td>Giant squid (<em>D. gigas</em>)</td>
<td>Water retention ability, color, textural and sensory attributes</td>
<td>10%</td>
<td>[62]</td>
</tr>
<tr>
<td>Sodium chloride, sugars, polyols</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Powder and liquid</td>
<td><em>Larimichthys polyactis</em></td>
<td>Water activity, VBN, color moisture content</td>
<td>Sodium chloride: 4%, Glucose: 10%, Glycerin: 10%</td>
<td>[63]</td>
</tr>
<tr>
<td>Starch, glycine, sodium lactate</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Powder and liquid</td>
<td><em>L. polyactis</em></td>
<td>Water activity, VBN, color, moisture content</td>
<td>Sodium lactate: 7.5%</td>
<td>[64]</td>
</tr>
<tr>
<td>Glycerol</td>
<td>—</td>
<td>Steamed</td>
<td>Liquid</td>
<td><em>Scomber japonicus</em>, and Brazilian sandperch (<em>Pseudopercis semifasciata</em>)</td>
<td>Water activity, textural and sensory attributes</td>
<td>20%</td>
<td>[65]</td>
</tr>
<tr>
<td>Na and Ca salts of polyuronides and carboxymethyl cellulose</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Gel-strengthening effects</td>
<td>2%–6%</td>
<td>[66]</td>
</tr>
<tr>
<td>L-ascorbic acid (AsA) and dehydro-L-ascorbic acid (DAsA)</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Powder</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Gel strength analysis</td>
<td>DAsA: 10 μg/g</td>
<td>[67]</td>
</tr>
<tr>
<td>Sodium-L-ascorbate (SA)</td>
<td>—</td>
<td>Steamed in Nojax cellulose casing</td>
<td>Powder</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>pH, textural and sensory attributes</td>
<td>0.2%</td>
<td>[68]</td>
</tr>
<tr>
<td>ω-3 fatty acids from algae Eicosapentaenoic acid, docosahexaenoic acid</td>
<td>Not mentioned</td>
<td>Heated in a water bath</td>
<td>Oil</td>
<td>Cod (<em>Gadus morhua</em>)</td>
<td>TBARS, fatty acid content, color</td>
<td>500 mg/85 g</td>
<td>[69]</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Oil</td>
<td>Walleye pollock (<em>T. chalcogramma</em>)</td>
<td>Microscopic observation, viscosity, gel-forming ability</td>
<td>10%</td>
<td>[70]</td>
</tr>
<tr>
<td>Common name</td>
<td>Species</td>
<td>Cooking method</td>
<td>Used as</td>
<td>Fish source for surimi</td>
<td>Metrics</td>
<td>Optimum amount or treatment condition</td>
<td>References</td>
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<tr>
<td>Eicosapentaenoic acid, docosahexaenoic acid</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Oil</td>
<td>Walleye pollock (<em>T. chalcogramma</em>), Threadfin bream (<em>Nemipteridae</em>), White croaker (<em>Genyonemus lineatus</em>), and Japanese jack mackerel (<em>Trachurus japonicus</em>)</td>
<td>Proximate analysis, color, water-holding capacity, physical attributes</td>
<td>5%–30%</td>
<td>[72]</td>
</tr>
<tr>
<td>ω-3 PUFAs-rich oils</td>
<td>Flaxseed, algae, menhaden, krill, blend (flaxseed : algae : krill, 8 : 1 : 1)</td>
<td>Heated in a water bath</td>
<td>Oil</td>
<td>Alaska pollock (<em>T. chalcogramma</em>)</td>
<td>Torsion test, and rheological attributes</td>
<td>9 g/100 g</td>
<td>[73]</td>
</tr>
<tr>
<td>Ethanolic Kiam wood extract (EKWE) + commercial tannin (CT)</td>
<td>Hopea sp.</td>
<td>Heated in a water bath</td>
<td>Overdried powder</td>
<td>Striped catfish (<em>Pangasius hypophthalmus</em>)</td>
<td>pH, VBN, TBARS, color, TCA-soluble peptide, moisture, protein contents, textural attributes</td>
<td>EKWE: 0.08% CT: 0.02%–0.04%</td>
<td>[74]</td>
</tr>
<tr>
<td>Oxidized phenolic compounds: ferulic acid (OFA), tannic acid (OTA), catechin (OCT), caffeic acid (OCF)</td>
<td>Cocos nucifera</td>
<td>Heated in a water bath</td>
<td>Freeze-dried powder</td>
<td>Sardine (<em>S. albella</em>)</td>
<td>Total phenolic, expressible moisture, TCA-soluble peptide, and protein contents, color, textual, rheological, and sensory attributes</td>
<td>CHE-E60: 0.125%</td>
<td>[75]</td>
</tr>
<tr>
<td>Oxidized phenolic compounds: ferulic acid (OFA), tannic acid (OTA), catechin (OCT), caffeic acid (OCF)</td>
<td>—</td>
<td>Heated in a water bath in polyvinylidenecasing</td>
<td>Solution</td>
<td>Mackerel (<em>Rastrelliger kanagurta</em>)</td>
<td>SEM, expressible moisture, protein content, color, textural and sensory attributes</td>
<td>OFA: 0.40%, OTA: 0.50%, OCF: 0.50%, OCT: 10%</td>
<td>[76]</td>
</tr>
<tr>
<td>Oxidized phenolic compounds: ferulic acid (OFA), tannic acid (OTA), catechin (OCT), caffeic acid (OCF)</td>
<td>—</td>
<td>Heated in a water bath in polyvinylidenecasing</td>
<td>Powder</td>
<td>Bigeye snapper (<em>P. tayenus</em>)</td>
<td>SEM, expressible moisture, protein, and free amino acid content, color, textural and sensory attributes</td>
<td>OFA: 0.20%, OTA: 0.05%, OCF: 0.15%, OCT: 0.05%</td>
<td>[77]</td>
</tr>
<tr>
<td>Zinc sulfate (<em>ZnSO₄</em>), sodium tripolyphosphate (STPP)</td>
<td>—</td>
<td>Heated in a water bath in polyvinylidenecasing</td>
<td>Powder</td>
<td>Yellow stripe trevally (<em>Sekaroides leptolepis</em>)</td>
<td>Expressible moisture, lipid, phospholipid, and protein content, Ca-ATPase activity, color, textural attributes</td>
<td>ZnSO₄: 60 μmol/kg + STPP: 0.5%</td>
<td>[78]</td>
</tr>
</tbody>
</table>

SEM: scanning electron microscopy; FTIR: Fourier-transform infrared spectroscopy; VBN: volatile basic nitrogen; TBARS: thiobarbituric acid reactive substances; TCA: trichloroacetic acid.
2.3. Plant Source Additives. The chicken plasma protein, egg white, and beef plasma protein are considered as the most effective protease inhibitors for surimi [17, 22, 23, 156]. However, the use of chicken plasma and beef plasma protein has been forbidden because of the occurrence of avian influenza and mad cow disease, respectively. In addition, higher concentrations of beef plasma proteins have also been associated with off-flavors, while egg white is expensive and has an unwanted egg-like odor [29, 156]. Additionally, vegetarians would not want to consume surimi-based products prepared with additives from animal or even seafood sources. Therefore, alternate food-grade additives are still desired to enhance the gel strength of surimi, without affecting the customer demand. Various natural additives derived from the plant sources have been briefly described below.

2.3.1. Legumes. The effects of vegetable protein content, moisture, heating, and setting conditions on the physical attributes of kamaboko were examined by Yamashita [39]. A firm gel was obtained at 60°C for kamaboko with soybean protein and at 80°C with wheat gluten. When the kamaboko gels were supplemented with 5% vegetable protein, the changes in jelly strength, softness, and expressible water of the kamaboko with wheat gluten were somewhat greater than those of the kamaboko with soybean protein. According to the results of Chung and Lee, the addition of plant proteins including soy protein isolate, lactoalbumin, and wheat gluten remarkably lessened the strength of non-animal protein-incorporated surimi gels [40]. The textural and sensory attributes of fiberized surimi gel products were categorized as an increase in overall textural desirability and an increase in gel strength. In another study, egg white and milk protein isolate showed higher water retention ability than whey protein concentrate, soy protein isolate, and lactoalbumin [41].

Protein isolates from legume seeds can be used as alternate protein additives for the quality improvement of surimi gels. Legume seed isolates comprise trypsin inhibitors and have been used as the protease inhibitor in the preparation of surimi and surimi-based products [42, 43]. Benjakul et al. reported higher protease inhibitory activities of inhibitor extracts from soybean and black cowpea seeds [42]. A reduced gel-degradation activity (modori) and a high thermal stability were reported. In another study, they reported inhibitory effects of proteinase inhibitor extracts from Bambara groundnuts (Voandzeia subterranea), pigeon pea (Cajanus cajan), and cowpea (Vigna unguiculata) on autolysis and gel-degradation activity (modori) of threadfin bream surimi [43]. The whiteness of surimi gels reduced slightly with the addition of proteinase inhibitor. Similar results were obtained by Oujifard and colleagues [44]. The Bambara groundnut protein extracts at a level of 0.25 g/100 g showed improved autolytic inhibition, deformation, and breaking force in surimi prepared by threadfin bream (N. bleekeri) [44]. However, a slight reduction in whiteness was observed at increasing levels of Bambara groundnut protein isolates. These studies show that the addition of Bambara groundnut protein extracts at a suitable level could serve as an alternative food inhibitor to enhance the gelling properties of surimi.

Plant protein isolates, mainly soy protein isolates, have been used in the surimi industry because of their safety and rational price [45]. Luo et al. indicated the legumin and vicilin, two main legume seed storage proteins, as binders and cogelling agents in surimi gels [45]. Protease inhibitors isolated from legume seeds not only can help to reduce the gel-degradation process in surimi but can also improve the surimi gel properties by acting as filler or binder. Luo et al. reported higher breaking force and quality characteristics of silver carp surimi when supplemented at a ratio of 10% soy protein isolate [46]. Cardoso et al. studied the effects of dietary fiber and microbial transglutaminase from chicory and pea on the rheological properties of protein paste from gilthead sea bream, hake, meagre, and seabass [47]. It was found that a high degree of protein denaturation boosted gel hardness while a low degree of protein denaturation created gels with high deformability. It shows that the addition of microbial transglutaminase could serve as a possible additive for gels of those species having lesser protein unfolding ability.

Kadre et al. studied the effects of black bean (Phaseolus vulgaris) and mung bean (Phaseolus aureus) protein isolates on gelling properties and proteolysis of sardines (Sardinella
Albella) surimi [48]. An increase in deformation, breaking force, and water-holding capacity, as well as a lower level of degradation, was observed while the whiteness of kamaboko gels reduced slightly. Therefore, mung bean or black bean protein isolates could be effectively used to retard the proteolysis in sardine surimi, leading to improved gel strength. Klomklao and Benjakul studied the effects of the partially purified trypsin inhibitor from adzuki bean on the gelling properties and proteolysis of threadfin bream (N. bleekeri) surimi [49]. An increase in autolysis and inhibitory activity against sarcoplasmic proteinases as well as an increase in deformation and breaking force of kamaboko gel was observed at increasing levels of trypsin inhibitor while gel whiteness decreased slightly.

2.3.2. Starch. Starch is widely used to make fish-paste products as it enhances elasticity and increases the weight of the products. In attempts to control thermal stability, stickiness, and/or wetness under different serving and storage conditions, the functional characteristics of surimi seafood products have been widely studied using modified starches. Starch is the second most abundantly used ingredient in the manufacturing of fish-paste products because of its higher water-holding ability and capacity to replace fish proteins partially while preserving the desired gel features at a reduced cost [69,157–161].

Kim et al. reported a positive correlation between the amount of added starch and the quality of the food products [162]. Konoo et al. studied the effects of adding starch and amyllose to amylopectin contents of starch on the gelation properties of frozen walleye pollack surimi [50]. The breaking strength of gel was not affected by the change in amyllose: amylopectin ratio. However, it increased as the content of amyllose increased in pregelatinized starch [51]. A lower packing effect was observed at 90°C which hypothesized that the gelatinization of starch in fish meat can be prevented at this temperature. A strong correlation between the amyllose to amylopectin contents and the textural and rheological properties of starch-containing surimi gels was also reported by Lanier et al. [134].

The addition of normal and modified potato or sweet potato starch resulted in reductions in the characteristic storage modulus of surimi soils during heating [52]. Surimi gels supplemented with potato starch showed the highest firmness and cohesiveness. Yoo reported the best textural properties of the sand lance (Ammodytes personatus) fish-paste products at a level of 8% potato starch [53]. These studies show that potato or sweet potato starch can also be used as a potent food additive for the production of surimi.

2.3.3. Rice/Rice Flour. Fish meat and wheat flour are the major ingredients used for the production of surimi-based products. Rice flour, however, can be an important ingredient to enhance the rheological properties of surimi-based products. Several attempts have been made to evaluate the potential of rice flour as an alternative of wheat flour in the preparation of surimi products [41]. The effect of rice flour addition methods and milling types on the sensory and rheological attributes of surimi products were studied by Cho et al. [54].

Roll-mill rice at a concentration of 10%–15% displayed higher gel strength and sensory properties which show that roll-mill rice had strong potential for replacing wheat flour. The surimi products containing rice flour showed similar rheological and sensory characteristics to those of a finest commercial surimi product. Hence, rice flour might be an effective alternative to wheat flour for high-quality surimi products.

To replace wheat flour, Kwon and Lee examined the quality characteristics of fried fish cakes containing rice flour [55]. The total content of corn starch and rice flour was 28.83% of the total content of fish cake dough. There were no noteworthy differences in the pH, moisture level, appearance, color, flavor, taste, and overall acceptance as compared to the control group. The addition of 50% rice flour to surimi-based products could be an effective way to increase the content of rice flour without decreasing texture acceptability. Yoon et al. optimized the content of water and rice flour in surimi-based products [56]. The surimi-based products manufactured under optimal environment were comparable in gel strength to the commercial products. However, higher sensory evaluation scores were observed compared to those of the commercial products. These studies advocate that the rice flour not only can be employed as an alternative to wheat flour but can also be used to enhance the quality of surimi and surimi-based products.

2.3.4. Potato Powder. The food additives extracted from potato and potato protease inhibitors used in the preparation of fish-paste products are discussed in Sections 2.1, 2.3.2, and 2.4.4.

2.4. Cryoprotectants and Humectants. To inhibit denaturation and to lessen the damage of gel quality during cold storage, cryoprotectants are usually added to surimi products. Polyunsaturated fatty acids, protein additives, polyols, sugars, amino acids, salts, and plant extracts are frequently used as cryoprotectants and humectants to avoid fluctuations in myofibrillar proteins promoted by freezing, storage, or thawing [163]. Mechanical properties of surimi gels can be improved by the addition of numerous hydrocolloids such as konjac, carrageenan, locust bean, xanthan gum, and different microbial transglutaminases during the preparation of surimi products [57,164–166]. In contrast, the addition of alginates has been reported to weaken surimi gels [69].

2.4.1. Saccharides. Xanthan is a nongelling polysaccharide produced by the aerobic fermentation of Xanthomonas campestris [167]. The property of xanthan to form highly viscous and stable solution at low levels makes it acceptable in the food industry [168]. Xanthan displays quite remarkable synergistic interactions with other nongelling polysaccharides of the galactomannan family, leading to increases in gel formation and viscosity [168,169]. The three commercially available galactomannans are tara gum, locust bean gum, and guar gum.

The impact of low methoxyl pectin on the mechanical properties of silver carp surimi gels was studied by Barrera et al. [58]. An increase in hardness, shear stress, and water-holding capacity of the surimi gel was observed while no
significant improvement in the mechanical properties was observed as compared to the control. Benjakul et al. reported the effects of prawn-shell chitosan on surimi prepared by barred garfish (Hemiramphus far) [59]. Prawn-shell chitosan at a level of 1% of the surimi gel showed an increase in gel-enhancing effect on the heat-induced gelation of myofibrillar proteins. The addition of microbial transglutaminase generally increases the deformation and breaking force of surimi gel. However, this effect was significantly retarded in the presence of prawn-shell chitosan, resulting in lower magnitudes of deformation and breaking force.

The viscoelastic properties and the thermal stability of low-grade squid (Dosisicus gigas) surimi were investigated by Iglesias-Otero et al. [60]. The konjac glucomannan aqueous dispersion at a level of 1% expressed the best rheological properties, suggesting that the konjac glucomannan aqueous dispersion may be used to enhance the quality characteristics of low-grade squid surimi gel. Ramirez et al. evaluated the effect of protein-hydrochlorides on the gel-forming ability of myofibrillar proteins [142]. The xanthan/locust bean gum, at a ratio of 0.25/0.75, showed a positive improvement in the mechanical attributes of surimi gels. Eom et al. investigated the impact of carrageenan on the gelation property of salt-based Alaska pollock surimi [61]. The addition of 1.5% KCl rather than 2% NaCl significantly enhanced the gelling property of κ-carrageenan-induced surimi gel and showed increased gel strength, breaking force, and whiteness values.

2.4.2. Salts. Salts help in protein-protein interaction and the addition of salt is critical during the processing of fish-paste products. However, the high levels of sodium in foods, and consequently human consumption of sodium, have become a global issue. The prime harmful effects of excess sodium intake are hypertension and increased blood pressure. Subsequently, these conditions lead to cardiovascular diseases, including instances of stroke, heart attack, and related diseases, as well as gastric cancer and osteoporosis [170–172]. Therefore, to reduce sodium intake levels in fish-paste products, Hwang et al. prepared the sodium-reduced fried fish cakes containing potassium as a substitute for sodium [1]. The quality characteristics of 30% sodium-reduced fried fish cakes were not notably different from those of full-sodium fried fish cakes; however, the addition of potassium changed the color and reduced consumer acceptance. To increase the consumer preference for sodium-reduced fried fish cakes, the use of different food additives might be advantageous.

The weak gel-forming ability and the strong fishy smell of the giant squid (D. gigas) make it undesirable for the manufacturing of surimi-based products. To overcome these problems, Choi and Kim used Amorphophallus konjac flour to enhance the quality characteristics of giant-squid surimi products [62]. The increasing levels of A. konjac flour showed increases in gel texture and water retention ability while a reduction in color and taste was observed as compared to the commercial surimi products. The incorporation of the seasoning ingredients, such as sweeteners, might be helpful in removing the fishy smell of D. gigas, ultimately improving the gel properties of giant-squid surimi. Cando et al. reported that the sensory and mechanical properties of surimi gels with reduced-NaCl contents can be improved by the application of 300 Mpa high hydrostatic pressures [63]. The gels made with lower-NaCl contents revealed stronger and stable networks as showed by the ones with higher-NaCl contents.

2.4.3. Water Activity. It has been reported that humectants had the greatest effect on lowering water activity (a_w), with the efficiency of the reduction in a_w value decreasing in the order of NaCl, sodium lactate, glycine, propylene glycol, and sorbitol when each of them was combined with other humectants [173]. Kim and Park reported the impact of humectants such as sodium chloride, sugars, and polyols to lower the water activity (a_w) of various model kamaboko gels [64]. The effect of sodium chloride on lowering water activity (a_w) was the highest among all of the examined treatments while glucose caused browning reaction on the surface of kamaboko. In another study, they examined the effect of starch, glycine, and sodium lactate in lowering the water activity (a_w) of model kamaboko gels [65]. Sánchez Pascua et al. reported that glycerol (15%–50%) was effective in reducing the water activity (a_w), in Brazilian sand perch (Pseudopercis semifasciata) and mackerel (Scomber japonicus marplatensis) [66]. Among the tested humectants, the efficiency of the reduction in water activity (a_w) was observed decreasing in the order of sodium lactate, glycine, and starch.

2.4.4. Polyuronides. The effect of sodium and calcium salts of carboxymethyl and polyuronides cellulose on the strengthening of kamaboko gels was investigated by Niwa et al. [67]. It was reported that the calcium salts of pectic acid, pectic acid, alginic acid, and carboxymethyl cellulose enhanced the breaking force of Alaska pollock surimi, whereas their sodium salts except Na-pectinate failed to increase the breaking force. The increase in the breaking force induced by calcium carboxymethyl cellulose vanished upon increasing the degree of substitution of hydroxyl groups to carboxymethyl groups. Furthermore, fine cellulose particles enhanced the breaking strain and breaking force and reduced the amount of expressible water but were unsuccessful in the case of coarser particles. The addition of potato starch can increase the effectiveness even in the presence of coarse particles of cellulose.

2.4.5. Ascorbic Acid. The addition of dehydro-L-ascorbic acid and L-ascorbic acid to Alaska pollock surimi increased the gel strength [68]. It was suggested that the positive effect of L-ascorbic acid on gel formation might be due to the oxidation of sulfhydryl groups in fish proteins. Lee et al. studied the effects of sodium-L-ascorbate on the gel-forming abilities of surimi prepared by Alaska pollock [69]. Sodium-L-ascorbate remarkably enhanced the gel firmness, cohesiveness, strength, and sensory properties of the fiberized products at a level of 0.2%. It directly influenced the surimi quality regardless of vacuum treatment, indicating that airborne oxygen was not important. Freeze-syneresis, stimulated by ascorbate during frozen storage, was lessened by the application of hydroxypropylated-modified starch.

2.4.6. Unsaturated Fatty Acids. The addition of nutritionally beneficial ω-3 fatty acids during surimi preparation could
enhance the gel strength and stability [70]. For the effective use of highly unsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in surimi-based products, Okazaki et al. studied the gel-forming properties of frozen walleye pollock surimi containing DHA and EPA [71]. They reported that, to achieve a good quality product, the vigorous agitation of surimi with fish oil is essential to allow the heat-induced gelation of its emulsified product, through the formation of fine oil droplets.

Fukushima et al. investigated changes in the physical properties of heat-treated surimi gels prepared from threadfin bream, walleye pollock, Japanese jack mackerel, and white croaker [72]. The breaking strain, breaking strength, and water-holding capacity of the heat-treated gels became greater as the amount of fish oil increased. Furthermore, surimi seafood was nutritionally enhanced with ω-3 polyunsaturated fatty acid- (PUFA-) rich oils isolated from natural sources such as algae, flaxseed, menhaden, blend, and krill [73]. The Alaska pollock surimi supplemented with ω-3 PUFA-rich oils showed improved protein fundamental textural properties, heat-induced gelation, and endothermal transitions. These studies show that the interaction of unsaturated fatty acids and surimi proteins could contribute to the improvement in gel properties without altering the textural attributes.

2.4.7. Plant Ethanol Extracts. The effect of commercial tannin and ethanolic Kiam wood extract on the gelling characteristics of ice stored mackerel (Rastrelliger kanagurta) surimi was investigated by Balange et al. [74]. During 12 d of iced storage, pH, TBARS, TCA-soluble peptide and trimethylamine (TMA) contents, as well as total volatile base (TVB), of mackerel mince increased while gel-forming ability, myosin heavy chain band intensity, and whiteness decreased consistently. Deterioration, lipid oxidation, and protein degradation proceeded as storage time increased. An increase in deformation and breaking force of surimi gel was observed with the addition of 0.30% commercial tannin or 0.15% ethanolic Kiam wood extract during the first 6 d of storage. Therefore, commercial tannin and ethanolic Kiam wood extract had not shown a gel-enhancing effect on mackerel surimi. Furthermore, Bumard and Benjakul investigated the effects of coconut husk ethanolic isolates on the gel-forming ability of sardine (S. albellus) surimi [75]. Breaking force increased with the increasing levels of coconut husk ethanolic isolates while a decrease in whiteness and no detrimental effect on the sensory attributes of surimi gel was observed. It was concluded that the addition of coconut husk extracts at a suitable concentration could enhance the gel strength of sardine surimi with increased acceptability.

2.5. Compound Additives. The effect of different oxidized phenolic compounds such as tannic acid, OTA; ferulic acid, OFA; caffeic acid, OCF; and catechin, OCT, on the gelling attributes of mackerel (R. kanagurta) surimi was studied by Balange and Benjakul [76]. Gels supplemented with 0.50% OTA, 0.40% OFA, 0.10% OCT, or 0.50% OCF showed increases in deformation and breaking forces while a decrease in the expressive moisture content and myosin heavy chain band intensity was observed. In another study, they investigated the effects of oxidized phenolic compounds on the gel-forming abilities of bigeye snapper (P. tayenus) surimi [77]. An increase in breaking force and deformation with a decrease in expressible moisture contents was observed. Gels supplemented with the oxidized phenolic compounds had a finer matrix with smaller strands. The physicochemical characteristics of natural actomyosin advocate that oxidized phenolics could trigger the induction of disulfide bond formation or the conformational changes and cross-linking through amino groups. Therefore, the addition of oxidized phenolic compounds at an optimum concentration could enhance the strength of surimi gel.

Yongsawatdigul and Piyaodhammaviboon reported an inhibition in autolysis of surimi and mince prepared by lizardfish (S. tumbil) caused by p-tosyl-L-phenylalanyl chloromethyl ketone and phenylmethanesulfonyl fluoride, indicating the involvement of myofibrillar-associated serine proteinase. Tropomyosin and myosin heavy chain proteins were mainly hydrolyzed, resulting in poor textural properties [26]. Arfat and Benjakul investigated the effect of zinc chloride (ZnCl₂) and zinc sulfate (ZnSO₄) on the gel-forming abilities of surimi produced by yellow stripe trevally (Selaroides leptolepis) [78]. The kamaboko gels with ZnSO₄ added up to levels of 60 μmol/kg showed increased deformation, whiteness, and breaking force, as well as highly denser and interconnected gels. Therefore, ZnSO₄ at a suitable concentration could enhance gel strength and whiteness of dark-fleshed fish surimi.

3. Improvement in Quality and Functionality of Fish-Paste Products

Various food additives from seafood (e.g., fish, invertebrates, and seaweed), plants (e.g., vegetables, fruits, and herbal medicines), mushrooms, animal sources, and functional materials used to improve the quality and functionality of fish-paste products are listed in (Table 2) and described below.

3.1. Seafood Additives. It has been reported that various types of seafood, namely, fish including dried anchovy (Engraulis japonicus) powder [79, 80], pufferfish (Lagocephalus lunaris) powder [88], and skate (Raja kenojei) powder [81, 82], and invertebrates including warty sea squirt (Styela clava) ground flesh [83], its freeze-dried tunic powder [84], omandungi (Styela plicata) ground flesh [85], shrimp (Acetes japonicus) powder [86], and seaweed such as green laver (Ulva spp.) [87], have been used to enhance the quality and functionality of fish-paste.

The boiled and dried Japanese anchovy (E. japonicus) is a popular fisheries product in Korea and Japan. As the flesh can be eaten together with bone, boiled and dried anchovy products are regarded as good sources of calcium [174]. Bae and Lee evaluated the properties of fried fish-paste with added anchovy (E. japonicus) powder containing a high amount of calcium [79]. The fish-paste containing 10% anchovy powder displayed the highest values of adhesiveness, hardness, and strength. In the overall acceptance of sensory
<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Cooking method</th>
<th>Used as</th>
<th>Fish source for surimi</th>
<th>Metrics</th>
<th>Optimum amount or treatment condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seafood additives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anchovy</td>
<td>Engraulis japonicus</td>
<td>Fried</td>
<td>Dried powder</td>
<td>Sea bream</td>
<td>Calcium content, color, textural and sensory attributes</td>
<td>1%-2%</td>
<td>[79]</td>
</tr>
<tr>
<td>Anchovy</td>
<td>Engraulis japonicus</td>
<td>Fried</td>
<td>Dried powder</td>
<td>Sea bream</td>
<td>Calcium content, color, textural and sensory attributes</td>
<td>5%</td>
<td>[80]</td>
</tr>
<tr>
<td>Skate</td>
<td>Raja kenojei</td>
<td>Fried</td>
<td>Hot wind-dried skin and cartilage (6:4) powder</td>
<td>Sea bream</td>
<td>Moisture content, color, textural and sensory attributes</td>
<td>3%</td>
<td>[81]</td>
</tr>
<tr>
<td>Skate</td>
<td>Raja kenojei</td>
<td>Steamed</td>
<td>Fermented flesh</td>
<td>Nemipterus virgatus</td>
<td>Amino acid, and moisture content, color, textural and sensory attributes</td>
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<td>[82]</td>
</tr>
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<td>Warty sea squirt</td>
<td>Styela clava</td>
<td>Fried</td>
<td>Ground flesh</td>
<td>Himeji (Frozen yellow tentacle)</td>
<td>Color, textural and sensory attributes</td>
<td>5%</td>
<td>[83]</td>
</tr>
<tr>
<td>Warty sea squirt</td>
<td>Styela clava</td>
<td>Fried</td>
<td>Freeze-dried tunic powder</td>
<td>Frozen Itoyori</td>
<td>Color, textural and sensory attributes</td>
<td>1%</td>
<td>[84]</td>
</tr>
<tr>
<td>Pleated sea squirt</td>
<td>Styela plicata</td>
<td>Fried</td>
<td>Grinded flesh</td>
<td>Himeji (Frozen yellow tentacle)</td>
<td>Color, textural and sensory attributes</td>
<td>15%</td>
<td>[85]</td>
</tr>
<tr>
<td>Shrimp</td>
<td>Acetes japonicus</td>
<td>Fried</td>
<td>Powder</td>
<td>Frozen sea bream surimi</td>
<td>Moisture content, color, textural and sensory attributes</td>
<td>5%</td>
<td>[86]</td>
</tr>
<tr>
<td>Green laver</td>
<td>Ulva spp.</td>
<td>Fried</td>
<td>Powder</td>
<td>Frozen sea bream surimi</td>
<td>Color, sensory attributes</td>
<td>5%</td>
<td>[87]</td>
</tr>
<tr>
<td>Pufferfish</td>
<td>Lagocephalus lunaris</td>
<td>Fried</td>
<td>Powder</td>
<td>Nemipterus spp.</td>
<td>Moisture, crude protein, lipid, color, textural and sensory attributes</td>
<td>5%</td>
<td>[88]</td>
</tr>
<tr>
<td>Maesaengi</td>
<td>Capsosiphon fulvescens</td>
<td>Fried</td>
<td>Freeze-dried powder</td>
<td>Frozen sea bream surimi</td>
<td>Color, textural and sensory attributes</td>
<td>5%</td>
<td>[89]</td>
</tr>
<tr>
<td>Red snow crab</td>
<td>Chinomecetes japonicus</td>
<td>Fried</td>
<td>Leg-meat powder</td>
<td>Frozen Alaska pollock (T. chalcogramma)</td>
<td>Physiochemical and sensory attributes</td>
<td>6%</td>
<td>[90]</td>
</tr>
<tr>
<td>Plant source additives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mulberry leaf</td>
<td>Morus alba</td>
<td>Fried</td>
<td>Powder</td>
<td>Sea bream</td>
<td>Color, texture, sensory attributes</td>
<td>0.5%</td>
<td>[91]</td>
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<td>Onion</td>
<td>Allium cepa</td>
<td>Fried</td>
<td>Ethanol extract</td>
<td>Cutlassfish paste</td>
<td>Moisture content, TBC, VBN, color, sensory attributes</td>
<td>3%</td>
<td>[70]</td>
</tr>
<tr>
<td>Lotus leaf</td>
<td>Nelumbo nucifera</td>
<td>Fried</td>
<td>Powder</td>
<td>Sea bream</td>
<td>Color, textural and sensory attributes</td>
<td>0.5%</td>
<td>[92]</td>
</tr>
<tr>
<td>Beetroot and Spinach</td>
<td>Beta vulgaris and Spinacia oleracea</td>
<td>Microwave in kamaboko shape mold</td>
<td>Fresh beet root, spinach dish</td>
<td>Not mentioned</td>
<td>Moisture, texture analysis</td>
<td></td>
<td>[93]</td>
</tr>
<tr>
<td>Citrus fruits</td>
<td>Citrus limon, C. junos, C. unshiu, Fortunella japonica var. margarita</td>
<td>Steamed</td>
<td>Ground flesh pulp without seeds</td>
<td>Min Daegu flesh</td>
<td>Color, textural and sensory attributes</td>
<td></td>
<td>Cumquat</td>
</tr>
</tbody>
</table>
### Table 2: Continued.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Cooking method</th>
<th>Used as</th>
<th>Fish source for surimi</th>
<th>Metrics</th>
<th>Optimum amount or treatment condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat bran + SiO₂</td>
<td><em>Avena sativa</em></td>
<td>Boiled</td>
<td>Powder</td>
<td>Frozen Alaska pollock surimi</td>
<td>Color, textural and physiochemical attributes</td>
<td>6 g Oat bran/100 g SiO₂</td>
<td>[95]</td>
</tr>
<tr>
<td>Yam</td>
<td><em>Dioscorea japonica</em></td>
<td>Fried</td>
<td>Powder</td>
<td>Pollock, squid, shrimp</td>
<td>Folding test, color, textural and sensory attributes</td>
<td>2%</td>
<td>[96]</td>
</tr>
<tr>
<td>Wolfberry/Chinese Goji</td>
<td><em>Fructus lyii</em></td>
<td>Fried</td>
<td>Powder</td>
<td>Sea bream</td>
<td>Textural and sensory attributes</td>
<td>3%</td>
<td>[97]</td>
</tr>
<tr>
<td>Red ginseng</td>
<td><em>Panax ginseng</em> C.A. Meyer</td>
<td>Fried</td>
<td>Powder</td>
<td>Not described</td>
<td>Color, lipid oxidation, sensory attributes</td>
<td>1%</td>
<td>[98]</td>
</tr>
<tr>
<td>Korean angelica root</td>
<td><em>Angelicae gigantis</em> Radix</td>
<td>Fried</td>
<td>Powder</td>
<td>Sea bream</td>
<td>Textural and sensory attributes</td>
<td>0.5%</td>
<td>[99]</td>
</tr>
<tr>
<td>Turmeric</td>
<td><em>Curcuma longa L.</em></td>
<td>Fried</td>
<td>Powder</td>
<td>Pollock, squid, shrimp</td>
<td>Color, rheological and sensory attributes</td>
<td>3%</td>
<td>[100]</td>
</tr>
<tr>
<td>Wasabi</td>
<td><em>Wasabia japonica</em></td>
<td>Fried</td>
<td>Freeze-dried powder</td>
<td>Silver pomfret (<em>Pampus argenteus</em>)</td>
<td>Color, TBC, viable cell count, textural and sensory attributes</td>
<td>1.8%</td>
<td>[101]</td>
</tr>
<tr>
<td>Wolfiporia extensa</td>
<td><em>Poria cocos</em></td>
<td>Fried</td>
<td>Powder</td>
<td>Sea bream</td>
<td>Color, textural and sensory attributes</td>
<td>3%</td>
<td>[102]</td>
</tr>
<tr>
<td><strong>Mushroom additives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Button mushroom</td>
<td><em>Agaricus bisporus</em></td>
<td>Fried</td>
<td>Chopped fresh</td>
<td><em>Argyrosomus argenteatus</em></td>
<td>Textural and sensory attributes</td>
<td>10%</td>
<td>[103]</td>
</tr>
<tr>
<td>Enoki mushroom</td>
<td><em>Flammulina velutipes</em></td>
<td>Fried</td>
<td>Chopped fresh</td>
<td><em>A. argenteatus</em></td>
<td>Textural and sensory attributes</td>
<td>5%</td>
<td>[104]</td>
</tr>
<tr>
<td>Shiitake mushroom</td>
<td><em>Lentinus edodes</em></td>
<td>Fried</td>
<td>Chopped fresh</td>
<td><em>A. argenteatus</em></td>
<td>Textural and sensory attributes</td>
<td>10%</td>
<td>[105]</td>
</tr>
<tr>
<td>King oyster mushroom</td>
<td><em>Pleurotus eryngii</em></td>
<td>Fried</td>
<td>Paste</td>
<td>Silver white croaker (<em>Pennahia argentata</em>)</td>
<td>Textural and sensory attributes</td>
<td>10%</td>
<td>[106]</td>
</tr>
<tr>
<td>King oyster mushroom</td>
<td><em>Pleurotus eryngii</em></td>
<td>Steamed</td>
<td>Paste</td>
<td>Cuttlefish (<em>Sepia esculenta</em>)</td>
<td>Textural, physiochemical, sensory attributes</td>
<td>40%</td>
<td>[107]</td>
</tr>
<tr>
<td><strong>Animal source additives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry chicken</td>
<td><em>Gallus gallus domesticus</em></td>
<td>Fried</td>
<td>Breast meat batter</td>
<td><em>Itroyi;</em> Japanese threadfin bream, (<em>Nemipterus japonicus</em>)</td>
<td>Chemical composition, color, fatty acid composition, TBARS, sensory attributes</td>
<td>7.46% or 14.93%</td>
<td>[108]</td>
</tr>
<tr>
<td><strong>Functional food additives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-chain cellulose</td>
<td>—</td>
<td>Boiled</td>
<td>Powdered cellulose</td>
<td>Alaska pollock surimi</td>
<td>Textural and rheological attributes</td>
<td>6%</td>
<td>[109]</td>
</tr>
<tr>
<td>Dietary fiber from ascidian tunic</td>
<td><em>Halocynthia roretzi</em></td>
<td>Boiled</td>
<td>Refined dietary fiber</td>
<td>Alaska pollock surimi</td>
<td>Color, textural, physiological, and sensory attributes</td>
<td>5%</td>
<td>[110]</td>
</tr>
<tr>
<td>Common name</td>
<td>Species</td>
<td>Cooking method</td>
<td>Used as</td>
<td>Fish source for surimi</td>
<td>Metrics</td>
<td>Optimum amount or treatment condition</td>
<td>References</td>
</tr>
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<td>-------------</td>
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</tr>
<tr>
<td>Fiber and/ω-3 oil</td>
<td>—</td>
<td>Heated in a water bath</td>
<td>Powdered fiber, ω-3 oil</td>
<td>Alaska pollock surimi</td>
<td>Textural and rheological attributes</td>
<td>Fiber: 6–10 g and/ω-3: 100 g</td>
<td>[111]</td>
</tr>
<tr>
<td>Flaxseed or salmon oil</td>
<td>Not described</td>
<td>Cooked in a water bath</td>
<td>Oil</td>
<td>Frozen Alaska pollock surimi (T. chalcogramma)</td>
<td>TBARS, color, textural and sensory attributes</td>
<td>2 g/100 g</td>
<td>[112]</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>Glycine max</td>
<td>Heated in a water bath</td>
<td>Oil</td>
<td>Frozen silver carp surimi</td>
<td>Color, textural attributes</td>
<td>Soybean oil: &gt;3%</td>
<td>[113]</td>
</tr>
<tr>
<td>Calcium powder of cuttlefish bone treated with acetic acid</td>
<td>Sepia esculenta</td>
<td>Heating in a water bath</td>
<td>Calcium powder</td>
<td>Alaska pollock surimi</td>
<td>Moisture content, color, textural and sensory attributes</td>
<td>0.09%</td>
<td>[114]</td>
</tr>
<tr>
<td>Propolis</td>
<td>—</td>
<td>Fried</td>
<td>Alcohol extract (100%)</td>
<td>Alaska pollock meat paste</td>
<td>Color, textural and sensory attributes</td>
<td>0.17%</td>
<td>[115]</td>
</tr>
<tr>
<td>Propolis</td>
<td>—</td>
<td>Fried</td>
<td>Alcohol extract (100%)</td>
<td>Sand lance (Hypotyphus dybowskii)</td>
<td>Acid and peroxide value, VBN, sensory attributes</td>
<td>0.2%</td>
<td>[116]</td>
</tr>
<tr>
<td>Cheonggukjang</td>
<td>Fermented Glycine max by Bacillus sp.</td>
<td>Fried</td>
<td>Powder</td>
<td>Sea bream</td>
<td>Color, textural and sensory attributes</td>
<td>2%</td>
<td>[117]</td>
</tr>
</tbody>
</table>

TBC: total bacterial count; VBN: volatile basic nitrogen; TBARS: thiobarbituric acid reactive substances.
evaluation, small and large size fish-paste with 1% and 2%, respectively, of added anchovy powder was preferred. The similar increasing trend of calcium intensity was observed by Bae et al. [80]. However, the fried fish-paste products containing 20% anchovy powder displayed the highest values of adhesiveness, hardness, and strength. Regarding overall acceptance in the sensory evaluation, the fried fish-paste containing 5% anchovy was preferred. The optimal amounts of added anchovy in the results from Bae and Lee and Bae et al. were different, which might have been due to the different drying methods and sizes of the anchovies used in each study [79, 80]. Despite the differences in the two studies, the results suggest that anchovy powder could be applied to fried fish-paste products to achieve high calcium contents.

Skate contains many essential fatty acids including linolenic acid, linoleic acid, arachidonic acid, DHA, and EPA [175]. Skate skin contains high percentages of collagen, protein, and calcium [176], while its cartilage is rich in chondroitin sulfate [177]. To improve quality and nutrient levels, Cho and Kim prepared fried fish-paste with skate (R. kenojei) powder (hot wind-dried skin and cartilage powder [6 : 4]) [81]. According to Park et al., preference testing using steamed fish-paste product with different levels of added 14-day-fermented flesh of skate showed significant increases in brownness, smoothness, and skate flavor scores [82]. The amino acid contents of fish cake samples with 20% skate added had the highest overall preference scores. Consequently, the addition of 20% skate powder was optimal for the steamed fish cake to improve its quality characteristics with higher protein contents.

Warty sea squirt (S. clava) aquaculture in the Masan area of Korea’s south coast contains abundant unsaturated fatty acids and essential amino acids in its flesh [178] and glycosaminoglycan in its tunic [179]. Warty sea squirt has a unique taste and distinct antioxidant and anticancer activities [180]. Fried fish-paste supplemented with 20% warty sea squirt (Korean name: miduduk) displayed improved quality and functionality [83]. Choi et al. reported a fried fish-paste containing freeze-dried byproduct of warty sea squirt (S. clava tunic) [84]. The hardness and strength of fish-paste increased with increasing amounts of tunic powder. For overall acceptance in sensory evaluation, a fish-paste supplemented with 1% S. clava tunic obtained a relatively higher score. The results suggested that S. clava flesh and tunic could be used for fish-paste products to improve their quality and functionality. Park et al. reported improvement in the functional properties of fish-paste, by adding S. plicata (Korean name: omandungi) [85]. Fried fish-paste containing 20% S. plicata indicated the highest values of adhesiveness, hardness, and strength. For overall acceptance in sensory evaluation, a fish-paste containing 15% S. plicata obtained the highest score.

Seo and Cho reported the preparation of fish-paste with added shrimp (A. japonicus) powder [86]. The hardness, springiness, and cohesiveness increased with the increasing concentration of shrimp powder. However, the brittleness and gumminess reduced. In the sensory evaluation, the fish-paste prepared with 5% shrimp powder was most preferred. Cho and Kim reported the preparation of fish-paste with added green laver (Ulva spp.) powder [87]. The hardness, springiness, and cohesiveness increased with the increasing concentration of green laver powder. However, the brittleness and gumminess decreased upon the addition of green laver powder. In the sensory evaluation, a fish-paste prepared with 5% green laver powder was preferred over other fish-pastes. These results suggest that green laver powder could be applied to fish-paste to improve its quality and functionality.

Pufferfish containing taurine, hydroxyproline, lysine, and glycine impart a characteristic taste to food [181]. To improve the taste of fish-paste products, Park prepared a fish-paste by adding green rough-backed pufferfish (L. lunaris) powder [88]. The hardness, strength, gumminess, springiness, and chewiness of the fish-paste increased depending on pufferfish powder content. The preparation of fish-paste with added Capsosiphon fulvescens powder was reported by Park [89]. The hardness, springiness, strength, and cohesiveness increased with the increasing concentrations of C. fulvescens powder. However, the brittleness and gumminess reduced with the addition of C. fulvescens powder. In the sensory evaluation, overall, the fish-pastes prepared with 5% C. fulvescens powder were preferred over other fish-pastes. Thus, the results show that C. fulvescens powder could be used to create fish-paste products with high quality and functionality.

Kim et al. reported the changes in the sensory and physicochemical properties of a fish-paste containing red snow crab (Chionoecetes japonicus) leg-meat powder [90]. Hardness, gumminess, springiness, and cohesiveness increased with increasing levels of red snow crab leg-meat powder. Based on the sensory evaluation, it was concluded that the addition of red snow crab leg-meat powder at a level of 6% could improve the quality characteristics of fish-paste products.

3.2. Plant Source Additives. It has been reported that various plant sources, namely, vegetables (e.g., mulberry, beetroot, and spinach), fruits (e.g., citrus), and herbal medicines (e.g., Chinese matrimony vine, Korean Angelica root), have a significant effect in improving the quality and functionality of fish-paste products.

Mulberry (Morus alba) leaf has been used traditionally to treat a disease symptomized by thirst and stroke [182]. Ever since mulberry leaf was included as a food material in the Food Codex in 1988 by the Ministry of Food and Drug Safety (MFDS; Osong, Chungju, South Korea). Mulberry leaf powder has been used in various processed food products and health functional foods. Shin and Park reported the use of mulberry leaf powder in the preparation of fish-paste products [91]. In a texture meter test, the hardness increased, but the cohesiveness, springiness, gumminess, and brittleness decreased with increasing levels of mulberry leaf powder. In sensory evaluation, the fish-paste with 0.5% mulberry leaf powder revealed the highest acceptance scores in terms of flavor, texture, and overall quality.

Garden onion (bulb onion, Allium cepa) is a perennial plant belonging to Liliaceae and is widely used as a spice and seasoning vegetable in both the East and the West. Park et al. investigated the quality characteristics of fried fish-paste supplemented with flavonol-rich ethanol extract of onion [183]. In the sensory evaluation, as the amount of ethanol
extract of onion increased, so did the favorability in terms of flavor and taste. Notably, 3% ethanol extract of onion had the best score in overall acceptance. The results indicate that ethanol extract of onion can be used to prepare fried fish-paste products with high quality and functionality.

Shin reported the production of fish-paste with added lotus (Nelumbo nucifera) leaf powder [92]. The flavor, adhesiveness, and hardness increased with the increasing levels of lotus leaf powder. The fish-paste with 0.5% lotus leaf powder displayed the highest acceptance scores in terms of springiness, pleasant taste, appearance, texture, flavor, and overall quality. Thorat et al. prepared kamaboko containing green chili, coriander, ginger, garlic spice mixture, and ground beetroot or a spinach dish and then subjected the preparation to microwave cooking [93]. Kamaboko prepared with 10% beetroot and a 15% spinach dish was found to be superior to the others.

Yang and Cho developed a steamed fish cake with added 5% ground citrus fruits with skin [94]. The addition of citrus fruits did not disturb the flexibility of surimi. The pH of surimi samples increased in the following order: lemon (Citrus limon), citron (Citrus junos), tangerine (Citrus unshiu), kumquat (Fortunella japonica var. margarita), and control. The hardness of surimi was highest for lemon, followed by citron, tangerine, kumquat, and control surimi. In the sensory evaluation, surimi containing kumquat demonstrated higher scores in terms of color, taste, and textural properties. These results suggest that surimi could be prepared by adding citrus fruits to improve the quality and functionality.

Oat bran is a gluten-free dietary fiber that may decrease the risk of diabetes and heart diseases. The physicochemical properties of surimi gels supplemented with oat bran were studied by Alakhrash et al. [95]. The oat bran and SiO₂ incorporation (6 g/100 g) greatly improved water-holding capacity and gel texture while a reduction in whiteness was observed. Kim and Byun conducted tests on the sensory and physicochemical characteristics of fish-paste with added yam (Dioscorea japonica) powder [96]. The addition of yam powder increased gumminess, strength, springiness, and cohesiveness. In the sensory evaluation, the addition of 2% yam powder had the best scores in terms of taste, color, and overall preference.

Fructus lycii is a fruit produced by Lycium barbarum L. that has been used for nourishment, tonicity, and nourishment of the blood; it has antibacterial, anticancer, and antioxidant properties [182–186]. Shin et al. prepared fried fish-paste containing dried F. lycii powder [97]. In the textural analysis, cohesiveness increased, while brittleness and gumminess decreased, with increasing levels of F. lycii powder. The 3% F. lycii powder sample had the highest acceptance scores in terms of appearance, texture, taste, flavor, and overall acceptability.

Red ginseng-based fried fish-pastes containing different sizes and amounts of red ginseng powder were prepared and their biological properties, including lipid oxidation to improve fish-paste quality, were investigated [98]. The fish-paste products containing red ginseng powder showed a significant increase in hardness and chewiness. Furthermore, an inhibitory effect on lipid oxidation and reduced number of total microbes during storage were observed. These results suggest that high-quality fish-pastes could be achieved with the addition of 1% red ginseng powder, which effectively improved both sensory evaluation and physicochemical properties.

Angelicae Gigantis Radix (the dried root of Angelica gigas Nikai), more popularly known as Korean Angelica, is one of the widely used herbal medications [187]. It has been used in Korean medicine as an important medication for anemia and blood circulatory disorders. It has also been used for menstrual pains and postmenopausal syndromes. Shin et al. reported the development of fish-paste with A. gigas powder [99]. In a texture test, hardness, chewiness, and brittleness increased with increasing concentrations of Angelicae Gigantis powder. However, cohesiveness and springiness decreased. In the sensory evaluation, the fish-paste with 0.5% Angelicae Gigantis powder showed the highest acceptance scores for appearance, flavor, taste, texture, and overall quality.

Turmeric (Curcuma longa) has been used in Ayurvedic medicine from ancient times as a treatment for inflammatory conditions. It has been reportedly used for its various biological activities including antibacterial, antiviral, antifungal, antioxidative, and antiatherogenic effects [188]. Turmeric has been grown as a special crop in the central and southern areas around Jindo in South Korea [189]. Choi et al. investigated the sensory and rheological properties of fish-paste prepared with turmeric powder [84]. In terms of textural attributes, the addition of C. longa powder decreased springiness and improved strength. In the sensory evaluation, the addition of 3% C. longa powder was associated with the best scores for taste and overall preference.

Wasabi (Wasabia japonica) has various, beneficial health properties including antioxidative, antimicrobial, and antimutagenic activities [190, 191]. Jang et al. reported a high-quality fried fish-paste product made with silver pomfret (Pampus argenteus), which is one of the savory, soft, and delicious types of fish prepared by adding wasabi powder [101]. Notably, hardness, gumminess, and chewiness increased significantly with the addition of wasabi powder. In the sensory evaluation, 1.8% wasabi powder showed the best score in overall acceptability. These results show that wasabi could be used as a food additive or preservative in fish-paste products.

White Poria cocos wolf is the inner white part of P. cocos, a parasite found on Pinus densiflora. It is used to treat edema, chronic gastritis, gastric atony, nephrosis, acute gastroenteric catarrh, emesis, dizziness, and vomiting [192, 193]. Shin et al. prepared a fried fish cake with added white P. cocos powder and studied the textural and sensory characteristics [102]. In texture tests, brittleness was observed to increase, while springiness decreased, with increasing concentrations of P. cocos powder. The fish-paste product containing 3% white Poria cocos powder showed the highest acceptance scores for flavor, appearance, texture, taste, and overall quality.

Milk-vetch root is one of the most produced herbal medicines in Korea. It is a peeled and dried root of the herbaceous perennial herb known as Astragalus membranaceus, which belongs to the Fabaceae family [194]. It has been...
reported that milk-vetch root exerts diuretic, tonic, anti-hypertensive, hypoglycemic, immune-enhancing, antitumor, and antiviral effects [195]. Kim investigated the sensory and physicochemical properties of fish-paste prepared with milk-vetch root powder [196]. The strength, cohesiveness, brittleness, and gumminess of the fish-paste increased, while its springiness decreased, with an increasing amount of milk-vetch root powder. In the sensory evaluation, the addition of 1.0% milk-vetch root powder indicated best scores for taste, texture, color, and overall preference.

3.3. Mushroom Additives. Mushroom is a nutritional and functional food, as well as a vital source of physiologically beneficial medicines. Mushrooms have been used as traditional medicines in Korea, Japan, China, and other Asian countries for curing various diseases, including lymphatic disease, gastroenteric disorder, oral ulcer, and various cancers [197]. It has been reported that edible mushrooms in Korea number approximately 350 species [198]. Several edible mushrooms that are highly preferred have been added to fish-paste to enhance their quality and functionality.

Ha et al. prepared a fried fish-paste product with added *Agaricus bisporus*, which is a product described as having a racy flavor and taste [103]. The elasticity, hardness, brittleness, and gumminess of fish-paste with the added mushroom increased; however no significant difference in strength was observed. Regarding overall acceptance in a sensory evaluation, a fish-paste supplemented with 10% *A. bisporus* mushroom showed the highest scores.

Enoki mushroom (*Flammulina velutipes*) is well known for its anticarcinogenic and blood pressure-reducing properties. To utilize its functional properties, enoki mushroom was added to fried fish cake [104]. The sample containing 15% mushroom received the highest values for strength, gumminess, and brittleness. In the sensory evaluation, the fish cakes with 5% mushroom obtained favorable scores for overall acceptance.

Shiitake mushroom (*Lentinus edodes*) is known for its high level of β-glucans. Son et al. investigated the effects of shiitake mushroom on the textural properties of fried fish cake [105]. The fish cake containing shiitake mushroom received the highest values for strength, hardness, gumminess, and brittleness. In the sensory evaluation, the fish cakes with 10% shiitake mushroom sample obtained the best score for overall acceptance.

Kim et al. prepared a fried fish cake using cultured king oyster mushroom (*Pleurotus eryngii*) and silver white croaker (*Pennahia argentata*) surimi to enhance its physiological effects [106]. In assessing its quality properties, fish cake to which 10% mushroom was added received the highest values for strength, hardness, gumminess, and brittleness. The effect of king oyster mushroom on the textural and physicochemical properties of steamed cuttlefish (*Sepia esculenta*) fish cake was investigated by Chung et al. [107]. The fish-paste with added king oyster mushroom paste revealed significant decreases in gumminess, cohesiveness, and hardness while the springiness increased with increasing concentrations of king oyster mushroom paste. On the sensory evaluation basis, the cuttlefish-paste supplemented with 30%–50% king oyster mushroom showed higher overall acceptability. In the studies by Kim et al. and Chung et al., the optimal amounts of king oyster mushroom differed, which might have been due to the different cooking methods and surimi used in each study [106,107].

3.4. Animal Source Additives. Jin et al. investigated the effect of chicken meat on the quality characteristics of Itoyori (*Japanese threadfin bream, Nemipterus japonicus*) surimi [108]. The physicochemical properties such as fatty acid composition, shear force, and gel characteristics were affected by substitution with spent laying hen meat batter. However, sensory characteristics were less affected by this substitution. A huge amount of waste in the processing of grass carp is discarded. To deal with this waste, Gao et al. studied the processing technology used for fish and mushroom paste with salted fish cubes, mushroom, soybean, and fermented soybeans [199].

3.5. Functional Food Additives. Functional food additives including dietary fiber, ω-3-rich oil, calcium additives, and propolis have been used in the preparation of fish-paste products to increase their quality and functionality. Western populations have an inadequate quantity of health beneficial dietary fiber in their diets. Besides fiber, most Western populations also consume an insufficient amount of ω-3 PUFAs, while their sodium consumption greatly surpasses the recommended maximum. Debusca et al. prepared Alaska pollock surimi fortified with commercial long-chain cellulose as a source of dietary fiber [109]. Fiber fortification, up to a level of 6%, improved both texture and color; a slight decline in these values was observed at levels of 8% fiber. An increase in gel elasticity and thermal gelation of the fish cake was observed.

Yook et al. prepared a fish-paste by adding dietary fiber extracted from ascidian (*Halocynthia roretzi*) tunic to enhance its physiological properties [110]. The hardness, gumminess, adhesiveness, shear force, and chewiness of the fish-paste improved with the incorporation of the ascidian dietary fiber. The fish-paste with 5% ascidian dietary fiber scored the highest and was generally preferred by sensory panels. Tolasa et al. reported that the oxidative stability and the uniform dispersion of ω-3 unsaturated fatty acids can be attained in a highly consistent surimi gel system without the use of antioxidants [200]. Surimi and surimi-based products are famous throughout the world. In fact, US consumption increased in the 1980s, while the rate leveled off thereafter. The nutrification of food products with ω-3 PUFAs increases the health benefits of food, consequently increasing their market demand. Pietrowski et al. prepared surimi seafood products nutritionally enhanced with ω-3 PUFAs [201]. Although the nutrification of ω-3 PUFAs indicated an increase in lipid oxidation, it was within limits acceptable to consumers. The color of surimi seafood fortified with ω-3 PUFAs generally improved but no effect on textural characteristics was observed.

Debusca et al. reported that the fortification of Alaska pollock surimi with either ω-3 oil or dietary fiber alone, or in combination, improved both the textural and rheological properties [111]. The ω-3 oil and fiber in combination revealed
greater gelation of surimi and a slight reduction in color properties, indicating their interaction with myofibrillar proteins. Thus, it was suggested that the ω-3 oils and fiber could be effectively used as a fortifying agent to prepare high-quality surimi products with nutritional benefits. Sell et al. prepared surimi franks fortified with salmon oil or flaxseed [112]. The textural properties showed differences between frank types, with the flaxseed franks being cohesive, less gummy, softer, and chewy while the sensory evaluation showed no significant differences.

Chang et al. determined the effects of soybean oil and moisture contents on the physical properties of surimi gels [113]. The increasing levels of soybean oil and moisture contents resulted in an increase in whiteness and reduction in the chewiness, hardness, and breaking force. Setting in combination with soybean oil improved the textural and color parameters of surimi gels, indicating that soybean oil could be used to improve the color and textural properties of surimi seafood products.

Kim et al. prepared boiled fish cake using acetic acid-treated cuttlefish bone as a calcium additive agent [114]. The results of sensory evaluation of texture and whiteness were similar to those without this supplementation. In the mineral content analysis of heat-induced surimi gel, calcium content increased depending on the increasing concentration of acetic acid-treated cuttlefish bone powder, while phosphorus content did not change. The optimal concentration of acetic acid-treated cuttlefish bone powder for the preparation of high-quality heat-induced surimi gel was 0.09%.

Kim et al. prepared Alaska pollock fried fish-paste supplemented with propolis [115]. The addition of propolis enhanced the antiosppling and antioxidative ability, as well as gel strength, of fried fish-paste. In the sensory evaluation, the addition of 0.17% propolis showed the best score in overall acceptability. In another study, Kim et al. later studied sand-lance (Hypoptychus dybowskii) meat paste prepared with propolis [116]. Similar to the previous report, the addition of propolis on the fried fish-paste showed higher antioxidative and antiosppling activities. The fried sand-lance meat paste prepared with 0.2% added propolis was the most acceptable. Furthermore, the bitter taste of the sand-lance meat paste was reduced by adding 2% sweet amber powder.

Cheonggukjang is an ancient Korean food prepared by fermented soybean. It contains high levels of dietary fiber, oligosaccharides, isoflavones, saponin, lecithin, phytic acid, and phenolic compounds, among others. Its many beneficial properties have been reported, such as thrombolytic, anticancer, antimicrobial, hepatoprotective, antioxidant, and cholesterol-lowering effects [202]. Park et al. reported the use of fish-paste containing cheonggukjang powder [117]. The increasing concentrations of cheonggukjang resulted in an increased value of springiness, cohesiveness, and hardness; however, a reduction in brittleness and gummyiness values of fish-paste was observed.

4. Shelf-Life Extension of Fish-Paste Products

Fish-paste products may easily spoil due to residual microbes that are not removed by sterilization during the manufacturing process, or by contamination in packaging or the distribution process. For such reasons, even vacuum-packed fried fish-paste products have a shelf-life of fewer than 10 days during cold storage, which is relatively short [121, 122]. Various efforts have been made to develop long-term storage solutions for fish-paste products via physical and chemical methods [118–122]. Although these methods were found to be very effective, for the long-term storage of fish-paste products, they require sensitive and complex manipulation and are costly.

The addition of appropriate food additives to fish-paste products, as an effective preservation protocol, is another strategy. Potassium sorbate is a typical synthetic food preservative and is commonly used in processed foods including fish-paste products. This material is effective in inhibiting the growth of various microorganisms, as it has a slight sterilizing effect. The use of this material is permitted to a concentration of less than 2.0 g/kg in processed fish meat products (Food Code, Ministry of Food and Drug Safety, Republic of Korea). According to the results of Walker, sorbates and sorbic acid exert a very low level of mammalian toxicity, even in chronic studies as up to 10% of the diet did not show any carcinogenic activity [203]. In addition, Thakur and Patel summarized the application of sorbates in the shelf-life extension of fish and fish-based products [204]. The physical and chemical methods, along with natural food preservatives, for the long-term storage of fish-paste products are listed in Table 3 and briefly described below.

4.1. Physical and Chemical Methods. Various efforts have been focused on developing long-term storage solutions for fish-paste products via physical methods, including high pressurization [118], microwave pasteurization [93], high hydrostatic pressure treatment [119], and even irradiation [118, 121]. In addition, it has also been reported that treatment with chlorine dioxide solution at an appropriate concentration, which is harmless to humans, could be used to prevent spoilage and to extend the shelf-life [122].

High hydrostatic pressure technology has gradually gained popularity in the food industry over the last two decades [205]. In 2013, the worldwide market for high hydrostatic pressure equipment was estimated to be $350 million and it is expected to grow. Besides high hydrostatic pressure technologies, several types of radiation have also been tested, including ultraviolet, microwaves, and gamma irradiation treatments. Radiation is generally used to control biological hazards in the production of fish-paste products and to prolong the shelf-life of such products [206, 207]. Gamma radiation exerts potent antimicrobial effects, whereas ultraviolet rays are effective for the surface, but not interior, sterilization of porous dried fish products (e.g., fish-paste products). However, gamma irradiation treatment demands large-scale facilities and higher costs [207, 208].

Miyao et al. reported that growth of the majority of the pathogenic microorganisms present in surimi was inhibited at a high pressure of between 300 and 400 Mpa [118]. Several pressure-resistant strains were isolated from surimi and were identified as Moraxella sp., Achromobacter sp., Streptococcus faecalis, and Corynebacterium sp. It was suggested that
Table 3: Natural food additives and physicochemical methods used to improve the shelf-life of fish-paste products.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Cooking method</th>
<th>Used as</th>
<th>Fish source for surimi</th>
<th>Metrics</th>
<th>Optimum amount or treatment condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical and chemical methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High hydrostatic pressure</td>
<td>—</td>
<td>High-pressure treatment or heat treatment in sample tube with vacuum packaging</td>
<td>—</td>
<td>Frozen Alaska pollock</td>
<td>Microbial activity</td>
<td>400 MPa</td>
<td>[118]</td>
</tr>
<tr>
<td>High hydrostatic pressure</td>
<td>—</td>
<td>Not cooked</td>
<td>—</td>
<td>Tuna fish paste, Mackerel paste with paprika, mackerel paste with garlic, mackerel paste alone, and salmon paste</td>
<td>Microbial activity</td>
<td>200 MPa</td>
<td>[119]</td>
</tr>
<tr>
<td>Co-60 Gamma rays</td>
<td>—</td>
<td>Grilled</td>
<td>—</td>
<td>Commercially available fish meat paste products</td>
<td>TBC, textural, sensory, microbial, physicochemical attributes</td>
<td>75 kGy</td>
<td>[120]</td>
</tr>
<tr>
<td>Co-60 Gamma rays</td>
<td>—</td>
<td>Fried</td>
<td>—</td>
<td>Commercially available fish meat paste products</td>
<td>TBC, pH, textural, microbial, physicochemical attributes</td>
<td>3 kGy</td>
<td>[121]</td>
</tr>
<tr>
<td>Chlorine dioxide (ClO₂)</td>
<td>—</td>
<td>Steamed</td>
<td>—</td>
<td>Commercially available fish meat paste products</td>
<td>VBN, TBARS, pH, microbial, physicochemical, sensory attributes</td>
<td>50 ppm</td>
<td>[122]</td>
</tr>
<tr>
<td>Natural food additives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red pepper ethanol extract (RPEE) and chopped fresh red pepper (CFRP)</td>
<td>Capsicum annuum, Phellodendron amurense, Eugenia caryophyllus, Pinus rigida, Bletilla striata, and Paeonia albiflora</td>
<td>Fried</td>
<td>Ethanol extract and chopped fresh one</td>
<td>Frozen Alaska pollock</td>
<td>TBC, sensory attributes</td>
<td>RPEE: 10%, CFRP: 5%</td>
<td>[123]</td>
</tr>
<tr>
<td>Ethanol extract (EE), and water extract (WE)</td>
<td>Galus gallus domesticus</td>
<td>Fried</td>
<td>Powder</td>
<td>Frozen Alaska pollock</td>
<td>Viable cell count, pH, VBN, biochemical attributes</td>
<td>EWL: 5% + SHMP: 0.1%</td>
<td>[125]</td>
</tr>
<tr>
<td>Egg white lysozyme (EWL) and/or sodium hexametaphosphate (SHMP), sodium pyrophosphate (SPP)</td>
<td></td>
<td>Fried</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapefruit seed extract</td>
<td>Citrus paradisi</td>
<td>—</td>
<td>Solution</td>
<td>Commercially available fish meat paste products</td>
<td>Proximate composition, textual, biochemical, rheological, sensory attributes</td>
<td>1,000 ppm</td>
<td>[126]</td>
</tr>
<tr>
<td>Common name</td>
<td>Species</td>
<td>Cooking method</td>
<td>Used as</td>
<td>Fish source for surimi</td>
<td>Metrics</td>
<td>Optimum amount or treatment condition</td>
<td>References</td>
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<tr>
<td>Cinnamon bark extract</td>
<td><em>Cinnamomum cassia</em></td>
<td>Fried</td>
<td>Extracted solution</td>
<td>Frozen Alaska pollock</td>
<td>Antimicrobial activity</td>
<td>Sprayed diluted extract (1:1)</td>
<td>[127]</td>
</tr>
<tr>
<td>Alginic acid hydrolysate</td>
<td>—</td>
<td>Boiled</td>
<td>Hydrolysate solution</td>
<td>Frozen Alaska pollock</td>
<td>Relative viscosity, pH, color, rheological attributes</td>
<td>0.3%</td>
<td>[128]</td>
</tr>
<tr>
<td>Chitosan hydrolysate</td>
<td>—</td>
<td>Boiled</td>
<td>Hydrolysate solution</td>
<td>Frozen Alaska pollock</td>
<td>Viable cell counts, rheological, sensory attributes</td>
<td>0.3%</td>
<td>[129]</td>
</tr>
<tr>
<td>Nisin (N) and/or sucrose fatty acid esters (SFE)</td>
<td>—</td>
<td>Steamed</td>
<td>Powder and solution</td>
<td>Frozen Alaska pollock</td>
<td>Viable cell count, antimicrobial activity</td>
<td>0%: 12.5 μg/g + SFE: 10 m/g</td>
<td>[130]</td>
</tr>
<tr>
<td>Piscicolin KHI</td>
<td><em>Carnobacterium malthalomaticum</em></td>
<td>Steamed</td>
<td>Solution</td>
<td>Cod fish</td>
<td>Inhibitory assay, protein content, antimicrobial activity</td>
<td>50 AU/g.</td>
<td>[131]</td>
</tr>
<tr>
<td>Zein and soy protein isolate (SPI) films containing green tea extract (GTE)</td>
<td><em>Zea mays</em>; <em>Glycine max</em>; <em>Camellia sinensis</em></td>
<td>Fried</td>
<td>Edible film (GC-WPI) with GTE</td>
<td>Commercially available fish meat paste products</td>
<td>TBARS, color, microbial, physical attributes</td>
<td>GTE: 1%</td>
<td>[132]</td>
</tr>
<tr>
<td><em>Gelidium corneum</em> (GC)-Whey protein isolate (GC-WPI) blend film containing grapefruit seed extract (GSE)</td>
<td><em>Gelidium corneum</em>; <em>Citrus paradisi</em>; <em>Bos taurus</em></td>
<td>Not mentioned</td>
<td>Edible film (GC-WPI) with GSE</td>
<td>Commercially available fish meat paste products</td>
<td>Water vapor permeability, microbiological analysis, sensory attributes</td>
<td>GSE: 0.1%</td>
<td>[133]</td>
</tr>
</tbody>
</table>

TBC: total bacterial count; VBN: volatile basic nitrogen; TBARS: thiobarbituric acid reactive substances.
damage to the cell membrane and degradation of ribonucleic acids occurred in the high-pressure-treated cells. Malicki et al. investigated the use of high hydrostatic pressure to prolong the shelf-life of traditionally manufactured fish-paste stored under refrigeration (4°C) for 6 weeks [119]. Neither bacteria nor molds or yeasts were detected in the high hydrostatic pressure-treated fish-paste samples at any time point analyzed, irrespective of the pressurization conditions. In conclusion, these studies revealed the effectiveness of high hydrostatic pressure to prolong the shelf-life of traditionally manufactured fish-paste stored under refrigerated conditions for up to 6 weeks.

Kim et al. reported a reduction in the total aerobic bacterial counts in the grilled fish-paste stored at 5°C and irradiated by gamma rays at a level of 2.5kGy or more [120]. Additionally, the treatment of gamma rays at a level of 7.5 kGy at 30°C showed a significant inhibition in aerobic bacterial growth. Cho et al. investigated the effect of Co-60 gamma irradiation on fried fish-paste and studied the physicochemical properties of fish-paste products stored at low temperature (3 ± 1°C) and room temperature (10–20°C) [121]. There was no obvious difference between the vacuum- and air-packed groups. The irradiation of 3 kGy extended the shelf-life of fish-paste up to 2 times at room temperature and 3–4 times at low temperature. In both studies, the irradiation treatment caused very little textural degradation and no effect on the sensory characteristics of the samples was observed.

Shin et al. investigated the effects of chlorine dioxide (ClO₃⁻) treatment on the physicochemical and microbial properties of fish-paste products [122]. After ClO₃⁻ treatment, fish-paste samples were individually packed and stored at 4°C. The pH and VBN values of fish-paste decreased with increasing ClO₃⁻ concentration. ClO₃⁻ treatment significantly reduced the populations of total bacteria, yeast, and mold during storage. In particular, treatment with 50 ppm ClO₃⁻ significantly decreased the total bacterial count the most among all ClO₃⁻-treated fish-pastes, showing that 50 ppm chlorine dioxide was the optimum concentration to prolong the shelf-life of fish-paste products.

4.2. Natural Food Additives. The use of natural food preservatives rather than chemical and synthetic food preservatives is of worldwide interest. It has been reported that several natural food additives could extend the shelf-life of cooked fish and fish products. Some examples include onion ethanol extract [183], egg white lysozyme [125], grapefruit seed extract [126], chitosan hydrolysate [129], cinnamon bark extract [127], and red pepper extract [123], as well as mixtures of lysozymes, sodium hexametaphosphate, and sodium pyrophosphate.

The shelf-life of fried fish-paste products prepared by adding red pepper ethanol extract was estimated by Yoon et al. [123]. The shelf-life of the fried fish-paste with added 10% red pepper ethanol extract and 5% chopped fresh red pepper was 2 to 3 days longer than that of the commercial fish-paste product, thus demonstrating the most effective preservation effects. According to Ahn et al., the extracts of Eugenia caryophyllus, Pinus rigida, Bletilla striata, and Paeonia albiflora exerted strong inhibitory effects on the growth of microorganisms isolated from putrefied fish-paste [124]. Notably, the ethanol extract was more effective than water extract in all tested microorganisms. The inhibition level of each extract was evident at 2,000 ppm ethanol in the fish-paste.

Kim et al. investigated the inhibitory effects of lysozymes, mixtures of lysozymes, and other antibacterial substances such as sodium pyrophosphate and sodium hexametaphosphate on bacterial growth in surimi products [125]. The lysozymes inhibited growth in most of the tested isolates and the mixture of antibacterial substances showed increased effects compared with those when they were used individually. A mixture of 0.5% sodium hexametaphosphate, 0.5% sodium pyrophosphate, and 0.05% lysozyme in imitation crab and kamaboko showed the highest inhibitory activity.

The stabilizing effects of grapefruit seed extract on fish-paste products were investigated by Cho et al. [126]. Textural properties decreased with increasing storage period. The treatment of fish products with grapefruit seed extract prolonged the deterioration of fish-paste product proteins during storage up to 4-5 days. The chemical, sensory, and rheological evaluation revealed that the grapefruit seed extract could be used as an effective additive to extend the shelf-life of fish-paste products.

The predominant bacterium in most of the isolated microorganisms from packed and unpacked spoiled fish-paste products is Bacillus sp. [127]. Notably, yeast and molds are not reported in the vacuum-packed products. A hydrolysate of alginic acid has antimicrobial activity, but it has not been used at industrial scale. The alginic acid at a concentration of 0.3% prolonged shelf-life of fish-paste products by 4 days at 15°C and inhibited the growth of Bacillus sp. isolated from fish-paste products [128]. According to the results of Cho et al., the chitosan hydrolysate made with chitosanase from Aspergillus oryzae ATCC 22787 revealed the strongest antimicrobial activity and inhibited the growth of Bacillus sp. isolated from fish-paste products [129]. The addition of chitosan hydrolysate at a concentration of 0.3% resulted in extended shelf-life of up to 6 days at 15°C. Jeong et al. reported that spraying cinnamon bark extract on the surface of the fried fish-paste products could inhibit the growth of spoilage bacteria and mold at room temperature and resulted in prolonged shelf-life [127].

Yamazaki et al. investigated the effects of nisin and sucrose fatty acid esters on the growth of spoilage bacteria in fish-paste products [130]. Nisin exerted antibacterial activity against Bacillus subtilis and Bacillus licheniformis in a liquid medium at 20°C and resulted in a longer shelf-life for fish-paste products. It was concluded that the addition of nisin could be used as a potential alternative method to prevent spoilage caused by spore-forming bacteria in fish-paste products. The bacteriocin produced by Carnobacterium maltaromaticum had the ability to inhibit both Enterococcus sp. and Leuconostoc sp., which reduce the shelf-life of fish-paste products during preservation [131]. The results showed that the purified bacteriocin, piscicolin KHI and/or nisin, significantly inhibited the growth of Leuconostoc mesenteroides and Enterococcus faecium and could be used as a food-grade preservative for kamaboko gels.
Sakai and Yamaguchi examined the possibility of inhibiting lipid oxidation in boiled fish-paste by adding yuzu skin to the surimi [209]. The heat-treated control kamaboko and yuzu skin-added kamaboko (citron kamaboko) were refrigerated at 0°C for 2 days, and, after heating the surimi, the malonaldehyde content in both kamabokes was reduced. These results suggest that the addition of citron skin suppressed lipid oxidation in fish-paste products.

Various packing materials and wrapping techniques have also been employed to keep fish-paste products fresh and free of contaminants for a longer period of time. Lee et al. investigated the processing conditions and quality stability of retort pouch fried-mackerel fish-paste during storage [210]. The mackerel fish-paste was ground with added ingredients, fried in soybean oil, cooled, vacuum-packed in a laminated plastic film, and finally sterilized at 120°C for 20 min in a hot water circulating retort. The reported method showed a good preservation for 100 days at 25 ± 3°C. The sensory evaluation showed no significant differences between the prepared fish-paste products and that of products in the market. Ha et al. examined the optimum storage conditions for maintaining the quality of the fried fish-paste in retort pouches [211]. Both hardness and gel strength increased with increasing sterilization temperature. On the other hand, no differences were observed in elasticity and water-holding capacity.

Lee et al. elucidated the antioxidative effects of soy and/or zein protein films containing green tea extract on the physiological properties of fish-paste products during storage [132]. The soy protein films showed an increase in yellowness, while a decrease in yellowness was observed with the zein films. The lipid oxidation was retarded at day 2 of storage by wrapping the fried fish-paste products with soy and zein protein films containing green tea extract. Lim et al. prepared a Gelidium corneum whey protein isolate (GC-WPI) blend film containing grapefruit seed extract and studied the effect of this film on pathogenic bacterial inhibition during storage [133]. The GC-WPI blend was effective in decreasing the populations of Salmonella typhimurium, Listeria monocytogenes, and Escherichia coli on films treated with 0.1% grapefruit seed extract. These studies suggest that packaging fish-paste products in plant-based protein films containing green tea or grapefruit seed extract could be beneficial to prolong shelf-life.

5. Conclusion

The production of surimi dates back to ancient times. However, advancement in surimi processing technology started in 1960 with the discovery of cryoprotectants, which were helpful in maintaining the gel quality and functionality of fish-paste for relatively longer periods of frozen storage [3]. As the market share of quality-conscious consumers rises, the demand for the use of natural additives rather than chemical ingredients for surimi products will continue to increase. According to Park et al., the worldwide production of surimi-related products reached around 800,000 MT by 2011-2012, while the main fish sources for surimi products include Pacific whiting, Alaska pollock, jack mackerel, Atka mackerel, southern blue whiting, northern blue whiting, and hoki [3].

Surimi is subdivided into high-grade (FA, SA, and A) and low-grade (KA, KB, and RA) types, based on the quality of the raw fish sources. Most of the high-grade surimi is sold in Japan for the production of kamaboko and other high-quality surimi products and in Korea for the production of premium crabsticks. Low-grade surimi is sold in Europe and the United States for the manufacturing of crab sticks and in Korea and Japan for the preparation of other fried fish products [3]. With the technological advancements in the surimi processing industry, it has been possible to use low-grade surimi for the production of fish cakes, fish balls, and other surimi products.

With increasing demand and new processing techniques (e.g., the pH-shift method), the use of other seafood resources, such as small pelagic species and giant squid, for the production of surimi and surimi products is possible [212]. Notably, the production of surimi products is cheaper in Korea than elsewhere, but industry professionals are always searching for the most inexpensive surimi seafood sources. Hence, the production of surimi from aquaculture fish, such as catfish, and carp is growing in demand.

Food additives from animal and seafood sources, such as fish, chicken, beef plasma proteins, and egg white, are considered the most effective protease inhibitors for surimi. However, with the outbreak of avian influenza, mad cow disease, undesirable resulting characteristics, and some religious constraints, there is limited use of these food additives. Additionally, vegetarians in some parts of the world would not wish to consume surimi-based products containing additives derived from animals or even from seafood sources. Hence, there is a need to identify more effective and alternative food-grade ingredients (e.g., plant sources, seaweed, and microalgae) to be used in the preparation of fish-paste products.

Surimi is mainly used for human consumption. As such, the challenges of production cost, composition, nutritional value, and the shelf-life of surimi products can never be neglected. This review has provided an overview of natural and synthetic food additives and preservatives used to enhance the quality, functionality, and shelf-life of fish-paste products. In addition, the improvements in the fish-paste product quality and functionality by various food additives from seafood, plants, mushrooms, animal sources, and functional materials were discussed.

For decades, surimi and surimi-based products have been well known in East Asian countries such as Japan and Korea. However, with advancements in technology, they are attracting attention in other Asian as well as European countries. With the increasing consumption worldwide, the manufacturing and processing of fish-paste products may require new and improved additives to enhance their acceptability in the market. Continuous scientific innovations and improved processing technology will aid in further advancements and improvements in this area.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article and regarding the funding received.
Authors’ Contributions
Khawaja Muhammad Imran Bashir and Jin-Soo Kim contributed equally to this work.

Acknowledgments
This research was a part of the Project no. PJT200885, entitled “Development and Commercialization of Traditional Seafood Products Based on the Korean Coastal Marine Resources,” funded by the Ministry of Oceans and Fisheries, South Korea.

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Development of a Chemically Defined Medium for Better Yield and Purification of Enterocin Y31 from Enterococcus faecium Y31

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Received 31 March 2017; Revised 17 July 2017; Accepted 2 August 2017; Published 6 September 2017

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The macro- and micronutrients in traditional medium, such as MRS, used for cultivating lactic acid bacteria, especially for bacteriocin production, have not been defined, preventing the quantitative monitoring of metabolic flux during bacteriocin biosynthesis. To enhance Enterocin Y31 production and simplify steps of separation and purification, we developed a simplified chemically defined medium (SDM) for the growth of Enterococcus faecium Y31 and production of its bacteriocin, Enterocin Y31. We found that the bacterial growth was unrelated to Enterocin Y31 production in MRS; therefore, both the growth rate and the Enterocin Y31 production were set as the index for investigation. Single omission experiments revealed that 5 g/L NaCl, five vitamins, two nucleic acid bases, MgSO$_4$⋅7H$_2$O, MnSO$_4$⋅4H$_2$O, KH$_2$PO$_4$, K$_2$HPO$_4$, CH$_3$COONa, fourteen amino acids, and glucose were essential for the strain’s growth and Enterocin Y31 production. Thus, a novel simplified and defined medium (SDM) was formulated with 30 components in total. Consequently, Enterocin Y31 production yield was higher in SDM as compared to either MRS or CDM. SDM improved the Enterocin Y31 production and simplified the steps of purification (only two steps), which has broad potential applications.

1. Introduction

Bacteriocins are ribosomally synthesised antimicrobial proteins or protein complexes that inhibit other bacteria, and they either have a narrow antimicrobial spectrum (against the same species) or broad antimicrobial spectrum (against across genera) [1, 2]. Bacteriocin produced by lactic acid bacteria (LAB) is generally regarded as safe (GRAS) and Qualified Presumption of Safety (QPS) status [3, 4], and it is a desirable trait as a food additive for extending the shelf-life of the fermented food and controlling the safety of foods [5, 6]. Based on their molecular structure, bacteriocins can be divided into four groups: class I bacteriocins contain posttranslational modifications (lantibiotics and molecular weight ≤ 5 kDa); class II bacteriocins are unmodified and include small, heat-stable nonlanthionine-containing peptides [7]. The third class includes large heat-labile bacteriocins. Finally, the fourth class comprises complex bacteriocins containing a protein moiety with one or more other lipids or carbohydrates [8]. Most bacteriocins produced by the Enterococcus faecium group of LAB are class II bacteriocins. One subgroup, class IIa, contains bacteriocins with certain sequence motifs in their N-terminal halves and is active against the food pathogen, Listeria monocytogenes [9]. Class IIa bacteriocins have become a focus of research owing to their low molecular weight, good dispersity, and high thermostability. This is also a case of Enterocin Y31 produced by E. faecium Y31 in the current work [10]. Enterocin Y31 is a class IIa bacteriocin produced by E. faecium Y31 [11]. Previous research results showed that Enterocin Y31 had broad spectrum inhibition activity against Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922, Listeria monocytogenes ATCC 19111, Bacillus cereus CICC 20463, Staphylococcus aureus, and Salmonella enterica serovar
2. Materials and Methods

2.1. Strains and Growth Conditions. The bacteriocin-producer strain *E. faecium* Y31 isolated from Chinese traditional fermented foods (fermented vegetable juice from Lanzhou) was previously found to produce class IIa bacteriocin [10], fermented foods (fermented vegetable juice from Lanzhou) which was previously found to produce class IIa bacteriocin [10], and the growth of *E. faecium* Y31 with regard to application in foods are as follows: (1) bacteriocins are secreted in very small quantities in the production media; and (2) the media used to grow the bacteriocin-producing strains are very complex [12], as the LAB are fastidious organisms requiring amino acids, B vitamins, and various minerals for their growth. These components are normally provided by sources such as soybean meal, yeast extract, and meat extract. All of these sources contain proteins and peptides as sources of amino acids, which are often very closely related to the bacteriocin produced. Therefore, purification of bacteriocin from such a complex medium requires a series of steps. At each step, there is some loss, and sometimes after the final purification, the antimicrobial activity may be completely lost. Complex media do not allow the examination of all individual medium factors [13, 14] affecting bacteriocin production.

Ricciardi reported a modified chemically defined medium to promote the growth of *Lactobacillus casei* and *Lactobacillus plantarum* groups [15]. In the present study, we improved the chemically defined medium to improve the yield of Enterocin Y31 produced by *E. faecium* Y31. There are four objectives of this study: (1) to develop a new and improved production method for Enterocin Y31 (produced by *E. faecium* Y31), (2) to determine the macro- and micronutrients necessary for cultivating *E. faecium* Y31 and improving Enterocin Y31 production, (3) to develop a novel and simple separation and purification method for Enterocin Y31 (avoiding a series of chromatography steps and the loss of Enterocin Y31 activity), and (4) to compare Enterocin Y31 production in a defined medium with that in a complex medium. This is feasible using a completely defined medium, and a minimal medium for bacteriocin production can thus be established.

2.2. Medium. The growth of *E. faecium* Y31 and production of Enterocin Y31 in a defined medium in a fermenter were compared with those in MRS (reference medium) used for the optimisation of the growth of *E. faecium* Y31 and the production of Enterocin Y31. This medium CDM consisted of 44 components described by Khan et al. [12] (listed in Table 1). All chemicals used for the preparation of the defined medium were of analytical grade and obtained from either Sigma-Aldrich (St. Louis, MO, USA) or BDH Chemicals Ltd. (Poole, UK). For all the experiments, aqueous stock solutions of the individual components were prepared in the appropriate concentrations and sterilized by autoclaving, with the exception of the heat-sensitive components. The heat-sensitive amino acids (asparagine, glutamine, and tryptophan), all of the B vitamins, and FeSO₄ were filter-sterilized (pore size 0.22 μm membrane; Millipore Corp., Billerica, MA, USA). All the stock solutions were then stored at 4°C except FeSO₄ which was freshly prepared before each experiment because it is prone to oxidation during storage. In addition to the defined medium, MRS medium was used as a reference for comparison with the defined medium before each experiment.

2.3. Determination of the Antimicrobial Activity of Enterocin Y31. The antimicrobial activity of bacteriocin was monitored using the agar well diffusion assay (AWDA) as described previously [16] with some modifications. *E. faecium* Y31 was cultured for two or three generations, and then the cell-free supernatants (CFSs) of *E. faecium* Y31 were prepared by centrifuging for 30 min at 10000 g. Aliquots (80 μL) of the cell-free supernatants (CFSs) of *E. faecium* Y31 were added to the wells (6 mm diameter) on double-layer agar plates previously inoculated with 0.2 mL overnight culture of the indicator strain *E. coli* ATCC 25922. Plates were kept static at room temperature for 3 h to allow diffusion of the CFSs evenly in the agar, and then the plates were incubated for 24 h at 37°C. The bacteriocin titre was determined by eliminating the effect of the organic acid, eliminating the effect of H₂O₂, and protease verification. The diameter of the zone of inhibition (not including the wells) was measured using a Vernier caliper. An arbitrary unit (AU/mL) was defined as the reciprocal of the highest dilution that produced an inhibition zone. Specific activity was expressed as that unit per milligram of protein.

2.4. Protein Concentration Determination. Protein concentration was determined by the BCA (bicinchoninic acid) microassay method (Pierce, Rockford, IL, USA) [17].

2.5. Monitoring of the Growth of *E. faecium* Y31. The OD value of the fermentation liquid of *E. faecium* Y31 was monitored with UV spectrophotometry at 600 nm each hour. Sterile culture medium was used as blanks to subtract the background value. Samples having an OD600 nm value of more than 0.7 were diluted with the sterile culture medium, and the corrected OD value was obtained by multiplying the dilution factor. ODMax (600 nm) represents the maximum OD value of the growth of *E. faecium* Y31. ODMax (600 nm) was calculated before any centrifugation.

2.6. Determination of the Relative Growth Rate. The relative growth rate was calculated according to Monod [18]. The following equation was used to calculate the growth rate:

\[ \mu = \left( \frac{\ln \text{OD}_2 - \ln \text{OD}_1}{t_2 - t_1} \right) \mu_{\text{CDM}}, \]  

where \( \mu \) is the relative growth rate (h⁻¹), ln is the symbol for natural logarithm, and \( \text{OD}_1 \) and \( \text{OD}_2 \) are the corrected OD.
### Table 1: Composition of CDM and SDM medium.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>CDM</th>
<th>SDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids (g L(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>L-Cys</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Proline</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>L-Serine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>L-Valine</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>Nucleic acid bases (g L(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine·SO(_4)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Guanine</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Uracil</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Thymine</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Orotic acid</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>B vitamins (mg L(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Para-aminobenzoic acid</td>
<td>10.00</td>
<td>—</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Ca-pantothenic acid</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Cyanocobalamin</td>
<td>2.00</td>
<td>—</td>
</tr>
<tr>
<td>Thiamine</td>
<td>10.00</td>
<td>—</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>20.00</td>
<td>—</td>
</tr>
<tr>
<td>Minerals (g L(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese sulfate</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>0.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Ferrous sulfate.7H(_2)O</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>7.00</td>
<td>7.00</td>
</tr>
</tbody>
</table>

### Table 1: Continued.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>CDM</th>
<th>SDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon source (g L(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Others (g L(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Glycerol</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NaCl</td>
<td>—</td>
<td>5.00</td>
</tr>
</tbody>
</table>

2.7. Effects of NaCl, CaCl\(_2\), and Glycerol on the Growth of *E. faecium Y31* and Enterocin Y31 Production. To determine the effects of these components, 5 g/L, 10 g/L, 15 g/L, and 20 g/L NaCl, 10 g/L CaCl\(_2\), and 50 g/L glycerol were individually added to 1000 mL CDM, and CDM medium with no additions was used as a control. The inoculum size was 2%, fermentation temperature was 37 °C, and inoculation time was 24 h. The culturing liquid was then centrifuged for 20 min at 10,000 × g, and then the CFSs were used to determine Enterocin Y31 activity and OD max (24 h).

2.8. Effects of Individually Omitting Each Amino Acid, B Vitamin, Mineral, and Nucleic Acid Base on the Growth of *E. faecium Y31* and Enterocin Y31 Production. According to the Leave One Out (LOO) technique, each amino acid, B vitamin, mineral, and nucleic acid base was sequentially and individually omitted from 1000 mL CDM medium, and CDM without additions was used as a control. The inoculum size was 2%, fermentation temperature was 37 °C, and inoculation time was 24 h. The culture liquid was then centrifuged for 20 min at 10,000 × g, and then the CFSs were used to determine Enterocin Y31 activity and OD max (24 h).

The following terms were used to describe the relationship between each medium component and growth, as determined by the single omission technique. A constituent was considered essential if its omission resulted in less than half the maximum strain growth rate and production of bacteriocin of the positive control, stimulatory when its absence resulted in the fact that the growth rate and the bacteriocin production was between 50% and 80% of that observed in complete CDM, and nonessential if the growth rate and Enterocin Y31 production was 80% (or more) of that obtained in the complete CDM.

2.9. Purification of Enterocin Y31. *E. faecium* Y31 was allowed to grow in the SDM (Table 1) for 24 h. The cells were then removed by centrifugation (11,800 × g, 25 min). The CFSs were then filtered through a 0.22 μm filter (low-protein-binding HVLP filter; Millipore Corp.) to remove any remaining cells. These filtered CFSs were referred to as crude bacteriocin. The crude bacteriocin was subjected to ultrafiltration of 1 kDa and 30 kDa. The retentate from the 1 kDa step was then subjected to Tricine-SDS-PAGE.

2.10. Statistical Analyses. All experiments were performed separately three times, and mean ± standard deviation (SD) values were calculated from triplicate determinations. All
3. Results and Discussion

3.1. Relationship between the Growth of the Strain and Enterocin Y31 Activity. The growth of *E. faecium* Y31 was unrelated to Enterocin Y31 activity in MRS medium, as shown in Figure 1. The result was consistent with the result of Bacterocin J23 produced by *Lactobacillus paracasei* J23 as described by Huaxi Yi in our research group (not published), which was inconsistent with the result of Salivaricin produced by *L. salivarius* CRL 1328 [19]; thus, the growth rate of *E. faecium* Y31 and Enterocin Y31 activity were used as detection indexes for developing a novel chemical defined medium. Enterocin Y31 activity could be detected at the exponential phase of the producing strain (9 h) and reached its highest value of 11.26 ± 0.36 mm at stationary phase of producing strain (24 h). ODmax remained stable at 16 h of fermentation of strain Y31; thus, ODmax (16 h) was used as an index of the strain growth status.

3.2. Effect of NaCl Concentration on the Growth of *E. faecium* Y31 and Enterocin Y31 Production. The effect of NaCl at 5 g/L, 10 g/L, 15 g/L, and 20 g/L was determined in CDM with CDM with no additives as the control. The results indicated that the growth of *E. faecium* Y31 and production of Enterocin Y31 were not significant difference when adding 5 g/L or 10 g/L NaCl and 0 g/L and 15 g/L NaCl had no effect on the growth of strain Y31 and production of Enterocin Y31. When the concentration of NaCl was 20%, the growth of *E. faecium* Y31 and production of Enterocin Y31 reduced, which showed the significant difference when compared to 0 g/L and 15 g/L NaCl. We can conclude that 20 g/L NaCl have a negative effect on the growth of strain Y31 and the production of Enterocin Y31. When the adding concentration of NaCl was 5 g/L, the ODmax (16 h) of *E. faecium* Y31 was highest 1.72 ± 0.01, and the antibacterial activity also reached the maximum value 1283.64 ± 41.40 AU/mg protein. Based the significant difference analysis, we could conclude that 5 g/L NaCl stimulated the growth of strain Y31 (*P* < 0.05) and had no effect on the production of Enterocin Y31 (*P* < 0.05) whereas 10 g/L, 15 g/L, and 20 g/L NaCl had no effect on the production of Enterocin Y31 but had a negative effect on the growth of *E. faecium* Y31 (*P* < 0.05), whereas adding 50 g/L glycerol had a slight negative effect on the growth of *E. faecium* Y31 (*P* < 0.05) and a negative influence on Enterocin Y31 production (*P* < 0.05).

3.3. Effect of CaCl₂ and Glycerol on the Growth of *E. faecium* Y31 and Enterocin Y31 Production. The results shown in Table 2 indicated that adding 10 g/L CaCl₂ to CDM had no effect on the growth of *E. faecium* Y31 but had a negative effect on the production of Enterocin Y31 (*P* < 0.05), whereas adding 50 g/L glycerol had a slight negative effect on the growth of *E. faecium* Y31 (*P* < 0.05) and a negative influence on Enterocin Y31 production (*P* < 0.05).

3.4. Effect of Individually Omitting Each Amino Acid, B Vitamin, Mineral, and Nucleic Acid Base on the Growth of *E. faecium* Y31 and Enterocin Y31 Production. According to the Leave One Out (LOO) technique, each amino acid, B vitamin, mineral, and nucleic acid base was sequentially omitted in a series of prepared formulations, along with CDM as a control. The results shown in Table 3 indicated that L-Arg, L-Gly, L-His, L-Leu, L-Met, L-Phe, L-Thr, L-Ser, and L-Cys are essential amino acids which had an effect on the growth of *E. faecium* Y31 and Enterocin Y31 production, and the stimulatory amino acids mainly included L-Asn, L-Glu, L-Ifu, L-Iso, L-Lys, and L-Lys. Omitting the stimulatory amino acids resulted in the slow growth of *E. faecium* Y31 and low production
Table 2: Effects of CaCl$_2$ and glycerol on the growth of Enterococcus faecium Y31 and Enterocin Y31 production.

<table>
<thead>
<tr>
<th>Added components</th>
<th>ODmax (600 nm) after 16 h</th>
<th>Enterocin Y31 production (AU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (CDM)</td>
<td>1.69 ± 0.03$^b$</td>
<td>1242.24 ± 41.41$^c$</td>
</tr>
<tr>
<td>10 g/L CaCl$_2$</td>
<td>1.70 ± 0.02$^b$</td>
<td>289.86 ± 41.41$^b$</td>
</tr>
<tr>
<td>50 g/L glycerol</td>
<td>1.53 ± 0.03$^a$</td>
<td>565.91 ± 41.81$^b$</td>
</tr>
</tbody>
</table>

Note: CDM is explained in Table 1, and means in the same row not sharing a common superscript letter are significantly different from each other ($P < 0.05$).

Table 3: Effect of omitting a single amino acid on the growth of Enterococcus faecium Y31 and Enterocin Y31 production.

<table>
<thead>
<tr>
<th>Omitted amino acid</th>
<th>ODmax (16 h)</th>
<th>Relative growth rate</th>
<th>Enterocin Y31 production (AU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (CDM)</td>
<td>1.69 ± 0.03$^b$</td>
<td>100</td>
<td>1242.24 ± 41.41$^d$</td>
</tr>
<tr>
<td>L-Ala</td>
<td>1.50 ± 0.05$^g$</td>
<td>90</td>
<td>1228.43 ± 63.25$^c$</td>
</tr>
<tr>
<td>L-Arg</td>
<td>0.76 ± 0.02$^c$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Asp</td>
<td>1.43 ± 0.04$^d$</td>
<td>93</td>
<td>1173.22 ± 63.25$^d$</td>
</tr>
<tr>
<td>L-Asn</td>
<td>0.91 ± 0.03$^d$</td>
<td>60</td>
<td>1228.43 ± 82.25$^{cd}$</td>
</tr>
<tr>
<td>L-Cys</td>
<td>1.00 ± 0.04$^{de}$</td>
<td>44</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Glu</td>
<td>1.52 ± 0.04$^g$</td>
<td>72</td>
<td>1104.20 ± 23.91$^c$</td>
</tr>
<tr>
<td>L-Gln</td>
<td>1.71 ± 0.04$^b$</td>
<td>84</td>
<td>1242.24 ± 56.86$^d$</td>
</tr>
<tr>
<td>L-Gly</td>
<td>0.61 ± 0.03$^{ab}$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-His</td>
<td>0.66 ± 0.03$^{bc}$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Isol</td>
<td>1.06 ± 0.04$^{ef}$</td>
<td>79</td>
<td>662.52 ± 82.51$^b$</td>
</tr>
<tr>
<td>L-Leu</td>
<td>0.71 ± 0.03$^{bc}$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Lys</td>
<td>1.16 ± 0.04$^f$</td>
<td>80</td>
<td>621.11 ± 41.40$^b$</td>
</tr>
<tr>
<td>L-Met</td>
<td>0.53 ± 0.02$^a$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Phe</td>
<td>0.77 ± 0.02$^c$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Pro</td>
<td>1.81 ± 0.03$^b$</td>
<td>71</td>
<td>1104.20 ± 65.45$^{cd}$</td>
</tr>
<tr>
<td>L-Ser</td>
<td>0.49 ± 0.03$^a$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Thr</td>
<td>0.68 ± 0.02$^{bc}$</td>
<td>n.g.</td>
<td>—$^a$</td>
</tr>
<tr>
<td>L-Try</td>
<td>1.12 ± 0.03$^{ef}$</td>
<td>80</td>
<td>573.22 ± 23.91$^c$</td>
</tr>
<tr>
<td>L-Tyr</td>
<td>1.70 ± 0.07$^b$</td>
<td>100</td>
<td>1200.82 ± 34.87$^{cd}$</td>
</tr>
<tr>
<td>L-Val</td>
<td>1.96 ± 0.08$^i$</td>
<td>98</td>
<td>1228.43 ± 65.82$^{cd}$</td>
</tr>
</tbody>
</table>

Note: “n.g.” represents that the strain did not grow and “—” represents that there was no inhibition activity. CDM is explained in Table 1, and means in the same row not sharing a common superscript letter are significantly different from each other ($P < 0.05$).

of Enterocin Y31. Therefore, the essential amino acids and stimulatory amino acids were included in the SDM (listed in Table 1). A previous study reported the similar results, as Zhang et al. found that six amino acids (arginine, histidine, isoleucine, leucine, methionine, and valine) were necessary for the lactococci, enterococci, and streptococci [20].

Pyridoxal and pantothenic acid are essential B vitamins which had an effect on the growth of E. faecium Y31 and Enterocin Y31 production, and the stimulatory B vitamins mainly included niacin and riboflavin. Wherein the stimulatory B vitamins could not result in the growth arrest of E. faecium Y31 and the biosynthesis arrest of Enterocin Y31, they could cause low growth rate and low biosynthesis volume. Therefore, the essential and stimulatory B vitamins were needed in the SDM. Omitting folic acid would cause a reduction of 80% in the ODmax (16 h) of E. faecium Y31; therefore folic acid is also needed for the growth of E. faecium Y31 and the production of Enterocin Y31 (Figure 3). It has been reported that nutritional requirements of L. delbrueckii ssp. lactis CRL 581 growth were nicotinic acid and pyridoxal and pantothenic acid, whereas the growth of L. delbrueckii ssp. lactis CRL 654 requires niacin and pantothenic acid, and riboflavin and cyanocobalamin were found to be stimulatory for both strains [21]. Research results about S. thermophilus strains (ST1, ST7, ST8, ST11, ST18, and ST21) indicated that riboflavin was essential for the growth of all strains, whereas Ca-pantothenic acid and nicotinic acid were also required [22].

In the present study, MgSO$_4$$\cdot$7H$_2$O was an essential mineral and MnSO$_4$$\cdot$4H$_2$O, KH$_2$PO$_4$, K$_2$HPO$_4$, and CH$_3$COONa were stimulatory minerals which had an effect on the growth of E. faecium Y31 and Enterocin Y31 production (Figure 4). The necessity of magnesium and phosphate has previously been reported by other researchers for various LAB species [20, 23, 24].

Adenine and uracil are stimulatory nucleic acids which had an effect on the growth of E. faecium Y31 and Enterocin
Y31 production (Figure 5). No nucleic acid was essential for the growth of Enterococcus faecium Y31 and Enterocin Y31 production.

3.5. Comparison of Enterocin Y31 Production on MRS Medium and SDM. A novel chemical defined medium (SDM) was developed (Table 1 and Figures 3, 4, and 5), and SDM contained NaCl, L-Arg, L-Gly, L-His, L-Leu, L-Met, L-Phe, L-Thr, L-Ser, L-Cys, L-Asn, L-Glu, L-Iso, L-Lys, L-Try, pyridoxal, pantothenic acid, niacin, riboflavin, folic acid, MgSO$_4$·7H$_2$O, MnSO$_4$·4H$_2$O, KH$_2$PO$_4$, K$_2$HPO$_4$, CH$_3$COONa, adenine, and uracil. Strain Y31 grew better on SDM than on MRS medium (Figure 6). As shown in Figure 7, Enterocin Y31 production on SDM was 1.6 times than that on MRS.

3.6. Purification and Characterization of Enterocin Y31. After the removal of cells from the defined medium, the cell-free supernatant was subjected to ultrafiltration through a 30 kDa ultrafiltration membrane. The active permeate liquor was then subjected to a second ultrafiltration step using a 1 kDa NMWL. The antimicrobial activity was again found in the retentate (10240 AU/L). Enterocin Y31 was purified by Tricine-SDS-PAGE and its molecular weight was 6.74 kDa (shown in Figure 8).

4. Conclusion

When culturing Enterococcus faecium Y31 using novel chemical defined medium (SDM) Enterocin Y31 production increased 1.6 times than that on MRS. The ingredients were composed of NaCl, L-Arg, L-Gly, L-His, L-Leu, L-Met, L-Phe, L-Thr, L-Ser, L-Cys, L-Asn, L-Glu, L-Iso, L-Lys, L-Try, pyridoxal, pantothenic acid, niacin, riboflavin, folic acid, MgSO$_4$·7H$_2$O, MnSO$_4$·4H$_2$O, KH$_2$PO$_4$, K$_2$HPO$_4$, CH$_3$COONa, adenine, uracil, and glucose, which were the macro- and micronutrients needed by biosynthesis of Enterocin Y31. SDM was helpful to the growth of Enterococcus faecium Y31 and Enterocin Y31 production improvement. In addition, SDM avoided a series of chromatography steps and the loss of Enterocin Y31 production.
Figure 6: Growth of Enterococcus faecium Y31 on MRS and SDM.

Figure 7: Enterocin Y31 productions on MRS and SDM.

Figure 8: Tricine-SDS-PAGE of Enterocin Y31. M: protein marker; 1: Enterocin Y31.

Chemically defined medium has better potential for improving the bacteriocin production and reducing the steps of separation and purification. Chemically defined media is useful for purification of not only large bacteriocins such as Enterocin Y31, but also small peptide bacteriocins. The alternative use of a chemically defined medium meets the nutritional requirements of strains producing bacteriocins and therefore is an excellent alternative to reduce the load of contaminating peptides from the medium, thereby decreasing the purification steps. To our knowledge, no such studies have been conducted to improve class Ia bacteriocin Enterocin Y31 production and purify Enterocin Y31.

Ethical Approval

This study complies with the research ethics guidelines and it does not contain any studies with human or animal subjects.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

This work was financially supported by the 60th Financial Grant from China Postdoctoral Science Foundation (Grant no. 2016M602534), the Youth Innovation Talents Project of General Higher School in Guangdong Province (2016KQNCX144), and the National Natural Science Foundation of China (Grants nos. 31571850, 31771988, and 31701621).

References


Lipid Oxidation, Color Changes, and Microbiological Quality of Frozen Beef Burgers Incorporated with Shirazi Thyme, Cinnamon, and Rosemary Extracts

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Received 3 January 2017; Revised 17 March 2017; Accepted 11 April 2017; Published 21 May 2017

Academic Editor: Moreno Bondi

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In this study, the oxidative stability of beef burgers incorporated with Shirazi thyme, cinnamon, and rosemary extracts was compared with that of BHT-incorporated and antioxidant-free samples. The chemical composition, TBARS, metmyoglobin, pH, color, and microbial and sensory characteristics were evaluated during storage at −18°C for 2 months. The results indicated that Shirazi thyme and cinnamon extracts did not change the colorimetric properties significantly (P < 0.05). Incorporating natural antioxidants led to a significant (P < 0.05) reduction in TBARS (36.58–46.34%) and metmyoglobin (16.25–18.47%) as compared to control. Except for the control sample, total microbial counts of burgers were lower than the maximum allowed limit. Burgers formulated with Shirazi thyme revealed the lowest amount of total count. Regarding the sensory characteristics, the overall acceptability of different samples decreased in the order of cinnamon > BHT > Shirazi thyme > rosemary > control. Finally, the results showed that these plant extracts can be utilized as an alternative to synthetic antioxidants in formulation of burgers.

1. Introduction

Meat and meat products are among the most important protein sources in the daily diet of people living in developed countries. Beef burger is almost the most popular meat product consumed by millions of people from all over the world. The common processes (such as mincing, cooking, and salt addition) applied in the production of burgers enhance the formation of reactive oxygen species; therefore, the resultant product is highly vulnerable to oxidation [1]. Lipid and protein oxidations have been reported as the principal reason for the decreased quality of burgers during storage resulting in decreasing the shelf life [2–4].

Application of antioxidants is the best strategy to prevent oxidation reactions [5]. Synthetic antioxidants such as BHT, TBHQ, and BHA have various adverse human health effects including allergy, headache, asthma, and dermatitis. Therefore, the application of natural antioxidants (such as herbal essential oils and extracts) is of interest and can be observed in a growing number of research works [6–8] (Aliakbarlu et al. 2016). Moreover, consumers are increasingly demanding for green-labeled food products such as those containing natural antioxidant. Natural compounds can be obtained from natural sources such as plants, fruits, vegetables, oil seeds, spices, team, honey, bee pollen, and cereals [9]. A lot of extracts have been approved as GRAS (Generally Recognized as Safe); that is, the addition of these chemicals or substances into food is considered safe by experts, and their application is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) [10].

The potential of natural antioxidants in preventing the lipid oxidation in different food products has been evaluated by many researchers around the world [11]. It has been reported that the extracts of kinnow rind, pomegranate rind,
and seed powders were significantly able to decrease the lipid oxidation in goat meat patties [12]. The effects of natural (sage and rosemary) and synthetic (BHT) antioxidants on protein oxidation, discoloration, and texture of refrigerated patties produced from pig liver have been studied by Estévez et al. [13]. Authors reported that the antioxidant properties of natural and synthetic antioxidants were similar. In treated samples, the increase in the carbonyl content as a result of oxidation was significantly \( P < 0.05 \) lower than control samples. Moreover, antioxidants could successfully protect heme molecules from degradation. Among different natural antioxidants, it has been reported that the extracts of Shirazi thyme, cinnamon, and rosemary reveal substantial antioxidant capacity [14].

The main objective of the current study was to compare the effects of Shirazi thyme, cinnamon, and rosemary extracts (denoted as natural antioxidants) with those of BHT on protein and lipid oxidations and physicochemical, microbial, and sensory characteristics of frozen beef burgers during storage. The results of this study may have potential implications in substituting the synthetic antioxidants with nature-made ones.

2. Materials and Methods
A schematic representation of beef burger production is shown in Figure 1.

2.1. Materials. Beef flanks (18% fat) from freshly slaughtered animals (24 hours post slaughter) were purchased from a local market (Shiraz, Iran) and transported to the laboratory in insulated polystyrene ice-boxes. The meat was ground (Meat Grinder, Philips, HR2743, Amsterdam, Netherlands) through a perforated plate with a hole diameter of 5 mm. Thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, USA), 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, St. Louis, MO, USA) and diphenyl-2-picrylhydrazyl (DPPH\(^{\cdot}\)) (Sigma-Aldrich, St. Louis, MO, USA), soy protein isolate (SPI, 90% protein, Sonic Co., India), onions, salt, spices, and rusk were also used in this study.

Figure 1: Schematic diagram of experimental design.
2.2. Plant Extracts. Shirazi thyme (Zataria multiflora), cinnamon (Cinnamomum zeylanicum), and rosemary (Rosmarinus officinalis) were purchased from a local grocery. The genus and species were certified by experts from the Herbarium of Biology Department (Shiraz University, Shiraz, Iran). Dried plants were powdered with a mill and then kept in polyethylene bags at room temperature before extraction.

2.2.1. Extract Preparation. Plant powder (20 g) was thoroughly mixed with boiling distilled water (500 mL) for 5 min followed by filtration through Whatman grade No. 1 filter papers and concentration in rotary evaporator (Buchi Rotavapor R, Switzerland) at 50°C. The plant extract was then thoroughly mixed with boiling distilled water (500 mL) for roughly 3 min followed by filtration through Whatman grade No. 1 filter paper and concentration in a rotary evaporator (Buchi Rotavapor R, Switzerland) at 50°C.

2.2.2. DPPH Radical Scavenging Activity. Antioxidant activity of natural extracts and BHT was calculated using DPPH free radical method described by Çam et al. [16]. The amounts of extracts, required to replace BHT, were calculated based on the IC50 of BHT. The required amounts of natural extracts should have equal antioxidant activity to BHT.

2.3. Beef Burger

2.3.1. Burger Preparation and Storage. The formulation of beef burger used in this study was composed of beef (70%), onions (13.4%), rusk (4%), water (8%), SPI (3%), salt (1%), black (0.3%), and red (0.2%) peppers. Ground meat, salt, water, and minced onion were mixed together. After that, SPI was added and then mixed for 15 min to obtain a homogenous mixture. Rusk and spices were added in the next step. Finally, natural extracts and/or BHT were incorporated into the homogenate and then mixing process was continued. The concentration of BHT was 100 ppm. A burger maker (9 cm internal diameter) was used to shape the mixture into patties of approximately 90 g and 5 mm thickness. Burgers (15 patties per treatment) were then packed in light-resistant polyethylene containers and frozen at −18°C. Analyses were performed during 15-day intervals for two months. Before analysis, the samples were thawed at +4°C and then hand-mixed for 30 s.

2.3.2. Chemical Analysis. Moisture, protein, fat, and ash contents of burgers were determined according to the AOAC methods [17]. Carbohydrate content was estimated by subtracting the total amounts of moisture, protein, fat, and ash from 100.

2.3.3. pH Measurement. Meat sample (10 g) was homogenized with 50 mL deionized water for 1 min. pH was measured at room temperature using a digital pH meter (Suntech TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold) through direct immersion of electrode into the mixture [17].

2.3.4. Instrumental Color Evaluation. Color attributes of beef burgers were measured by $L^*a^*b^*$ method described by Yam and Papadakis [18]. The values of $L^*$ (brightness), $a^*$ (redness-greenness), and $b^*$ (yellowness-blueness) were measured on the whole outer surfaces of beef burger. A wooden box ($50 \times 50 \times 60 \text{ cm}^3$) equipped with a natural daylight source (6500 K) and a digital camera (Canon Powershot A540 of six megapixels resolution) in a vertical position and a distance of 25 cm from the samples was used for taking photos. Adobe Photoshop® CS6 was applied to determine the average surface color.

2.3.5. Determination of Lipid Oxidation. Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS). Meat sample (4 g) was homogenized with 20 mL trichloroacetic acid solution (20% w/v) and then centrifuged at 3000g for 10 min. The supernatant (2 mL) was mixed with 2 mL thiobarbituric acid solution (0.1% w/v in double distilled water) followed by heating in a water bath at 100°C for 30 min and then cooling to room temperature. Therefore, TBARS were extracted in chilled atmosphere. The absorbance of each extract was measured at 520 nm in a spectrophotometer (spec 1650PC, Shimadzu, Japan). 1,1,3,3-tetraethoxypropane was used to develop the standard curve for TBARS assay. TBARS values were reported as mg of malonaldehyde per kg of beef burger [7].

2.3.6. Metmyoglobin Measurement. A modified method of An et al. [19] was used to measure the metmyoglobin content in raw beef burgers. The sample (4 g) was mixed in a homogenizer with 20 mL of phosphate buffer (0.04 M, pH 6.8) and then stored at 1°C for 1 h. The mixture was then centrifuged at 3500g and 4°C for 30 min. Finally, the supernatant was filtered through Whatman grade No. 1 filter paper. The absorbance of the solution was measured at 525, 572, and 700 nm. Metmyoglobin (%) was calculated according to the following equation:

$$\text{Metmyoglobin (\%)} = \left[ 1.395 - \frac{(A_{572} - A_{700})}{(A_{525} - A_{700})} \right] \times 100.$$  

Total Aerobic Bacterial Count. Total aerobic bacteria were quantified every 15 days using the method described by Aliakbarlu et al. (2016). Beef burgers (10 g) were aseptically transferred into individual stomaching bags containing 90 mL of sterile saline (0.9%) and homogenized for 2 min. Serial 10-fold dilutions were prepared in saline and 100 μL from appropriate dilutions was spread on the surface of plate count agar for total aerobic count (TMC) and then incubated at 35°C for 24 h.

2.3.7. Sensory Evaluation. Sensory analyses have been performed by 20 trained panelists from the Department of Food Science and Technology (Shiraz University). Panelists were both male and female in the age range of 23–28 years old. Panelists were selected based on their previous experiences in consuming traditional beef burgers. Moreover, they receive a preparatory session before the sensory test to train them how to describe all evaluated factors completely. Burgers were fried at 150°C in a forced draft oven to a core temperature of 72°C and kept warm in the oven for 3–8 min until the sensory testing [20]. Rectangular pieces of approximately
2 cm were cut from the center of each burger and then served at room temperature. Each panelist randomly evaluated three pieces of all formulations and asked to give a numerical value 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); and odor 1 (imperceptible) to 9 (extremely intense). Tap water was provided for panelists to rinse their mouth between different samples. At the end of evaluation, each panelist was asked to give an overall score from 1 (dislike very much) to 9 (like very much) for the overall acceptability of different formulations.

2.4. Statistical Analysis. A completely randomized block design with five treatments (including antioxidant-free (control) treatment and those incorporated with BHT, cinnamon, rosemary, and Shirazi thyme extracts) was used in this study. Experiments were performed during a 60-day storage period at 15-day intervals (0, 15, 30, 45, and 60). Independent blocks (developed from three different batches) were replicated three times at each sampling point. A two-way analysis of variance (ANOVA) and Duncan’s multiple range tests (SAS 8.0 software, SAS Institute, Inc., Cary, NC, USA) were performed to analyze the effect of treatments, storage period, and their interaction on the physicochemical, microbial, and sensory characteristics of beef burgers at a confidence level of 0.05. In the analysis models, the treatments, storage times, and their interaction were assigned as fixed effects and the replications as random effects.

The given scores of different sensory attributes were compared between the treatments using general linear model (GLM). Duncan’s multiple range tests were used for comparison of means at a confidence level of 0.05. Treatments and assessors were considered as main effects and the replications as random effects. Data was reported as mean ± standard error (SE).

3. Results and Discussion

3.1. Antioxidant Activity of Extracts. The IC50 values (defined as the concentration of an antioxidant required to reduce the initial DPPH concentration by 50% using DPPH free radical method described by Çam et al. [16]) of natural extracts (including Shirazi thyme, cinnamon, and rosemary) and synthetic BHT were 0.022, 0.373, 0.062, and 0.107 mg/mL, respectively. Therefore, the amounts of Shirazi thyme, cinnamon, and rosemary concentrated extracts required to replace 100 ppm BHT in formulation were 22, 73, and 28 mL per kg of beef burger, respectively. Cinnamon is a source of bioactive compounds such as cinnamaldehyde, eugenol, and coumarin [21]. The effect of cinnamon extract as direct scavengers of free radicals has been studied by Roussel et al. [22]. Carnosic acid and carnosol are the main bioactive compounds present in the rosemary extract [23]. According to Shariffifar et al. [24], the major chemical compounds present in Shirazi thyme were carvacrol (33.65%), thymol (37.59%), p-cymene (7.72%), γ-terpinene (3.88%), and β-caryophyllene (2.06%).

3.2. Physicochemical Properties of Beef Burger. The results indicated that the samples contained 59.13% moisture, 19% protein, 12% fat, 7.72% carbohydrate, and 2% ash.

3.3. Color Stability. When assessing meat, a consumer pays a great attention to its color, which, as a visual impression, is induced mainly by the presence of pigments but it also depends on tissue composition and meat structure. Hence, the color of fresh meat is an important quality parameter that determines a consumer’s response and decision to buy or not to buy that product at retail. Changes in L*, a*, and b* values of beef burgers during frozen storage are shown in Table 1. No significant difference (P > 0.05) was observed between the samples incorporated with natural antioxidants and those incorporated with BHT at production time (day 0), resulting in low ∆E values at early stages of storage. All of the formulations showed significant decrease in a* during frozen storage. Formulation incorporated with rosemary extract showed lower amounts of a* at the end of storage time. The significant decrease in a* values indicated the red color reduction of products during storage; this is despite the fact that BHT- and extract-incorporated treatment significantly reduced the Met-mb development ratio in comparison to control one (Figure 2). During the first 30 days of storage, L* values increased significantly (P < 0.05) then remained constant. During frozen storage, changes in the amounts of L* in cinnamon- (6.42%) or Shirazi thyme-incorporated samples (5.97%) were less than those in other formulations. Samples incorporated with rosemary extract showed the least redness (4.33).

A significant decrease in a* values of raw pork patties containing grape seed extract and bearberry has been reported over a 12-day storage by Carpenter et al. [25]; however, b* values (particularly of control samples) increased significantly (P < 0.05). Similarly, Nuñez de Gonzalez et al. [26] reported that a* value of beefs stored under refrigeration
Table 1: Effect of addition of Shirazi thyme, cinnamon, and rosemary extracts and BHT on $L^*$, $a^*$, $b^*$, and $\Delta E$ values of raw beef burgers during frozen storage at $-18^\circ C$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
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<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>$60.33 \pm 0.88$ g**</td>
<td>$58.00 \pm 0.58$ hi</td>
<td>$71.33 \pm 0.34$ abc</td>
<td>$71.67 \pm 0.34$ ab</td>
<td>$72.67 \pm 0.67$ a</td>
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<td>$12.29 \pm 0.80$ cd</td>
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</tbody>
</table>

** Data represent averages of three independent repeats ± standard errors.

*** Means with different letters are significantly different ($P \leq 0.05$).

*** For each storage time, the color difference ($\Delta E$) was calculated by comparing the color attributes of different formulations with those of control sample.

for 10 weeks decreased significantly ($P < 0.05$). The results of the current study showed that high antioxidant activity of natural extracts can be potentially used to develop natural color stabilizers, particularly for controlling $L^*$ and $b^*$ values in frozen beef burger.

As mentioned already, samples incorporated with BHT and natural extracts did not show any significant difference in $\Delta E$ value at the beginning of the storage time. However, at the end of storage, the lowest and highest changes in $\Delta E$ were observed in cinnamon-incorporated and control samples. Red meat color is an important property of visual appearance and could influence meat purchasing decisions more than any other quality parameters. The color of meat products and meat is influenced by metmyoglobin percentage in muscle. Initially, the myoglobin was changed into oxymyoglobin (light pink color), which could result in brighter red meat, and then oxymyoglobin was oxidized into metmyoglobin during storage [7].

3.4. Metmyoglobin Content Assay. The amount of metmyoglobin has been positively linked to the extent of protein oxidation in meat products. The heme complex of heme (in proteins) consists of iron in the ferrous state ($Fe^{2+}$) which turns into the ferric state ($Fe^{3+}$) via a process called autoxidation [27]. The effects of natural extracts on the changes of metmyoglobin content are shown in Figure 2. The maximum amount of metmyoglobin (74.26%) was measured in antioxidant-free samples. The presence of antioxidants resulted in a significant decrease (16.25–18.47%) in the amounts of metmyoglobin developed during storage which was attributed to the strong antioxidant activity. An et al. [19] evaluated the percent metmyoglobin of the pork jerky samples incorporated with different kimchi powder concentrations. It has been reported that the metmyoglobin contents (%) of the samples prepared with various kimchi powder levels (ranged from 81 to 83%) were significantly ($P < 0.05$) lower than that of control sample (85%) [19]. There was no significant difference between the amounts of metmyoglobin under the influence of natural extracts or BHT indicating that these natural extracts can be utilized as a substitute for BHT to prevent discoloration.

The myoglobin reactions with peroxides result in the formation of lipid oxidation promoting compounds [28]. The lowest amount of metmyoglobin was measured in the samples formulated with cinnamon extract indicating that it was more effective than the other antioxidants (either natural or synthetic) in preventing metmyoglobin formation. A reverse relationship between the metmyoglobin level and $a^*$ value was observed in this study. A decrease in $a^*$ values corresponding to the decreased redness of lamb meat as a result of myoglobin oxidation (metmyoglobin formation) has been reported previously [29, 30].

Metmyoglobin content increased during storage. The lowest increasing rate was observed in the samples incorporated with rosemary extract. The highest amount of metmyoglobin was observed in the control sample at the end of storage. Changes in the metmyoglobin content of control sample became significant after 15 days.
Table 2: Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde per kg) of beef burger during frozen storage at −18°C.

<table>
<thead>
<tr>
<th>Sample</th>
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</tr>
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</tr>
<tr>
<td>Control</td>
<td>0.12 ± 0.01*</td>
</tr>
<tr>
<td>BHT</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Cinnamon extract</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Rosemary extract</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Shirazi thyme extract</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>

*Data represent averages of three independent repeats ± standard errors.

3.5. Oxidative Stability. Initiation is the step in which a fatty acid radical is produced. Two notable initiators in oxidative stability are OH· and HOO·, which combines with a hydrogen atom to make water and a fatty acid radical. The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way. The reaction stops when two radicals react and produce a nonradical species. The lipid oxidation leads to produce of some components causing off-flavors and reduced nutritional quality such as malondialdehyde and 4-hydroxynonenal.

Thiobarbituric acid test can be used to determine the secondary products of lipid oxidation in different foods such as meat and its products [31]. Thiobarbituric acid reactive substances (TBARS) values of different formulations (as a function of storage time) are shown in Table 2. Except for control sample, the variations in TBARS values of the other formulations revealed a similar pattern. Due to the absence of antioxidant in formulation, the observed changes in TBARS values of control were significantly (𝑃< 0.05) higher than others (86.36% higher than rosemary). Natural extracts were as effective as BHT for preventing lipid oxidation in beef burgers. This observation indicated that natural herbal extracts are suitable replacers for synthetic antioxidant. It has been reported that rosemary oleoresin (77.04%) and a mixture of BHT and BHA (78.69%) was equally efficient in inhibiting the lipid oxidation of breakfast sausage containing 25% turkey meat [32]. These findings are also in good agreement with those of Mielnik et al. [33], who reported lower TBARS values after cooking of mechanically deboned turkey meat already stored in dark cold and treated with rosemary (88.34%) and grape seed extracts (83.83%). Sánchez-Escalante et al. [34] reported that incorporating rosemary essential oil into beef patties led to a significant (𝑃< 0.05) decrease (64.00%) in TBARS values during cold storage. The essential oil of rosemary was also effective in preventing (72.43%) the rancidity of heat-treated turkey meat products [35]. It has also been reported that rosemary extract addition into deboned poultry meat can protect (98.20%) the product from cooking-induced oxidation [36].

Regardless of sample type examined, TBARS values increased significantly during the frozen storage and reached to a maximum amount of 0.41 mg malonaldehyde per kg in antioxidant-free (control) sample after 60 days. At TBARS values of 2.0, off-flavors are definitely detectable, and the meat is considered to be unacceptable [37].

3.6. Changes in pH Values. Changes in pH values of beef burgers during frozen storage are shown in Figure 3. All formulations revealed relatively similar pH values just after production ranging from 5.88 to 5.93, coinciding with the ISIRI 2008. Mohamed and Mansour [38] similarly reported that no significant differences (𝑃> 0.05) were observed in the pH values of beef patties after incorporating natural herbal extracts.

A downward pattern of different rates was observed in the pH values of all formulations during frozen storage. As no microbial growth was expected during storage, the decrease in the pH values could be attributed to the microbial growth during thawing (by consuming sugar and producing organic acids). Control sample had the lowest pH (5.51) at the end of storage. Emiroğlu et al. [39] reported that the pH values of
all fresh ground beef patty samples treated with thyme and oregano generally decreased after sixth day of storage \( (P > 0.05) \).

Also the result showed that, to decrease pH, amount of lipid oxidation and metmyoglobin significantly increased. Lapidot et al. (2005) evaluated lipid peroxidation of grilled red turkey muscle (Donor Kabab) as affected by pH. In this study, they show indeed that lipid peroxidation and myoglobin of a real fast food (turkey Doner Kebab, shawarma) is significantly more rapidly oxidized at pH 3.0 than at pH 5.0.

3.7. Microbial Growth. As shown in Figure 3, total aerobic bacterial counts of antioxidants-incorporated formulations were not significantly \((P < 0.05)\) different. The highest amount of total count was observed in control sample (Figure 3). Shirazi thyme-incorporated beef burgers had the lowest microbial count which could be attributed to the high antimicrobial properties resulting from thymol and carvacrol. The antibacterial mechanism of thymol and carvacrol is the disruption of the cytoplasmic membrane, which raises its permeability and depolarizes its potential. Total count of frozen raw beef burger should be lower than 6 log cfu/g [40]. Except for the control sample, the microbial counts of beef burgers were lower than the maximum allowed count. A relatively good correlation was observed between the pH values and total aerobic counts. For example, control sample had the lowest pH and the highest microbial count. Rosemary extract (1000 ppm) inhibitory effect on the microbial growth of surface-applied beef steaks has been reported by Djenane et al. [41]. However, Sánchez-Escalante et al. [34] reported that rosemary did not affect the microbial counts of beef patties during storage (3 log cfu/g).

An increase in the microbial counts was observed during storage, but not beyond the standard range. As a rule of thumb in good manufacturing and hygienic practices during preparation of beef burger and its related products, rapid and proper storage is important for decreasing the microbial growth and enhancing the shelf life.

Vieira et al. [42] studied the effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. The result showed that psychrotrophic bacteria increased \( (1.38 \text{ log}_{10} \text{CFU/g}) \) significantly during 90-day storage.

3.8. Sensory Properties. Color, flavor, and texture are the most important sensory attributes which influence the acceptability of meat products by consumers [43]. Sensory evaluation results of cooked beef burgers are shown in Figure 4. Generally, incorporating Shirazi thyme, cinnamon, and rosemary extracts into beef burger formulations had no significant influence on the sensory properties. Although samples formulated with Shirazi thyme and cinnamon extracts with 69.44 and 71.67% acceptability obtained the highest taste scores by panelists \((P < 0.05)\). The antioxidant properties of natural extracts were the main reason for the increased sensory scores of natural extract-incorporated formulations through preventing the formation of oxidation-mediated off-flavors and off-odors (short-chain aldehydes and ketones) during storage. Moreover, the presence of pleasant volatile constituents in natural extracts (particularly in cinnamon and Shirazi thyme) led to an increased sensory score in comparison to the BHT-incorporated formulation.

A significant increase in the flavor scores of beef patties incorporated with antioxidant mechanically deboned poultry meat during frozen storage was similarly reported by Mohamed and Mansour [38].

4. Conclusion

This work aimed to study the possible replacement of synthetic antioxidant BHT with natural extracts (including Shirazi thyme, cinnamon, and rosemary extracts) in beef burger formulation. Different samples were prepared; then different characteristics such as protein and lipid oxidation and physicochemical, microbial, and sensory properties were evaluated during frozen storage. Herbal extracts could inhibit the lipid oxidation in formulated burgers. Moreover, it was demonstrated that natural herbal extracts were generally better than BHT in preventing the lipid and protein oxidations as well as improving the sensory attributes of beef burgers. Therefore, it can be concluded that natural extracts of Shirazi thyme (0.022 mg/mL), cinnamon (0.373 mg/mL), and rosemary (0.062 mg/mL) can be used as natural substitutes for BHT (0.107 mg/mL). This substitution may have potential implication for developing green-labeled meat product. In future studies, it would be informative to study the effect of mixed extracts and their possible synergistic or antagonistic effects in extending the shelf life of meat products.
Additional Points

Practical Applications. Beef burger containing natural antioxidant is one of the most important functional food for human consumption attributable to its nutritional properties and health effects. However, the nutraceutical properties of this food have improved due to alternative BHT with extracts. The present investigation demonstrated the effects of adding various natural antioxidants to the beef burger, so as to monitor the changes that occur to the physicochemical, antimicrobial, and sensorial properties of the beef burger.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research project was financially supported by Scientific Affairs Shiraz University and Department of Food Science and Technology, Shiraz University, Shiraz, Iran.

References


[37] E. TEC, “Meat technology update, 1-6, 2009”.


Optimization of Ultrasound Extraction of Cactus Pear (Opuntia ficus indica) Seed Oil Based on Antioxidant Activity and Evaluation of Its Antimicrobial Activity

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Received 20 February 2017; Accepted 9 April 2017; Published 27 April 2017

The purpose of the present study was to determine the optimal ultrasound conditions (amplitude level and time) for the extraction of cactus pear seed oil with the highest antioxidant activity using a closed system. Seed oil was analyzed for yield, antioxidant activity by ABTS and DPPH, and antimicrobial activity. Conventional extraction methods were assessed for comparison. Amplitude level significantly affected antioxidant activity in linear terms (p < 0.0001 DPPH and p < 0.001 ABTS, resp.) so, at lower amplitudes, the higher antioxidant activity was achieved. The optimum ultrasound extraction conditions were of 78% amplitude for 10 min and yielded antioxidant activity values of 66.25 mg AAE/100 g and 289 μmol TE/100 g for ABTS and DPPH, respectively. Compared with conventional extraction methods, ultrasound exhibited lower oil yield and antioxidant activity but had the potential to achieve comparable results if multiple ultrasound extractions are performed in the time needed by conventional methods. Seed oils showed similar antimicrobial activity despite the extraction method and were more effective against Escherichia coli. The results demonstrated that ultrasound can be an alternative extraction method of seed oils from fruits such as cactus pear.

1. Introduction

Cactus pear fruit (Opuntia ficus indica) is common in arid and semiarid regions around the world [1]. This fruit that is mainly consumed fresh in Mexico [2] is composed by pulp, peel, and seeds [3]. According to several studies cactus pear fruit has bioactive compounds [4, 5] with high antioxidant and antimicrobial activity [6, 7]. Some of these compounds are found in the seeds [8], which comprise 3 to 15% of the cactus pear pulp [9] and are usually considered waste after pulp processing [7]. Seeds also have a high content of oil (98.8 g/kg) [10] characterized by high levels of linoleic and oleic acids [7] and other components as phenols [11], all which may contribute to human health [12]. Currently, seed oils have been used as natural agents for food preservation [13], and many have exhibited antimicrobial and antioxidant activity; some of these oils are from pumpkin [14], apple [15], black cumin [16], and basil [17] among other seeds. Cactus pear seeds from Opuntia dillenii also have a high antioxidant activity derived from bioactive compounds such as polyphenols and polyunsaturated fatty acids [18]. Some polyunsaturated fatty acids have also been identified in seeds from Opuntia ficus indica [19, 20] implying that these seeds may also have high antioxidant activity.

Seed oil is usually extracted by means of conventional methods such as Soxhlet and maceration, using heat, agitation, or long extraction times [21]. Microwave, supercritical fluids, and ultrasonic assisted extraction are unconventional methods that exert a physical effect on the sample [22]. Ultrasound has been used to extract antioxidants from many food materials including seeds. Ultrasound and ultrasound-assisted extractions use sound waves to produce cavitation...
microbubbles that collapse violently in the sample and facilitate the release and extraction of several compounds [23–25]. Some researchers had evaluated ultrasound-assisted extraction, in an open system, using a sonicator probe directly on the liquid sample to obtain seed oil from flaxseed [26], Korean pine [27], and pomegranate [28]. The purpose of the present study was to optimize the extraction conditions of cactus pear seed oil using ultrasound in a closed system based on antioxidant activity and using response surface methodology. Yield extraction and antioxidant and antimicrobial activity were compared with conventional methods.

2. Materials and Methods

2.1. Sample. Green cactus pear (Opuntia ficus indica), Reyna variety, was provided by the Mexican Association CoMeN-Tuna (Consejo Mexicano del Nopal y la Tuna A.C. of Actopan, Hidalgo, México) in spring of 2012. The green cactus pear seeds were obtained after several washes with water that removed the pulp and residues. The seeds were left to dry at ambient temperature until they reached a moisture of 6.43%. After the seeds were crushed using an industrial mill (Cyclotec 1093, Tecator, Höganäs, AB, Sweden), the powder was passed through a mesh sieve to obtain a particle size of approximately 0.5 mm and then stored in sealed plastic bags at room temperature and dark conditions.

2.2. Ultrasound Extraction. Ultrasound (VCX-1500, Sonic & Materials, Inc. Newtown, CT, USA) at 1500 W, with a constant frequency of 20 kHz and a probe of 25 mm, was used for the extraction of green cactus pear seeds oil. Extraction from milled and sieved seeds (20 g) was carried out at an amplitude and time ranges of 80 to 90% and 5 to 15 min, from milled and sieved seeds (20 g) was carried out at an extraction of green cactus pear seeds oil. Extraction was performed using an industrial mill (Cyclotec 1093, Tecator, Höganäs, AB, Sweden), the powder was passed through a mesh sieve to obtain a particle size of approximately 0.5 mm and then stored in sealed plastic bags at room temperature and dark conditions.

2.3. Soxhlet Extraction. Soxhlet extraction was performed according to the AOAC [30]. Milled and sieved seeds (5 g), hexane (120 mL), and a universal fat extraction system (Büchi Labortechnik AG, Flawil, SG, Switzerland) were used.

2.4. Maceration Extraction. Milled and sieved seeds (10 g) were introduced in a previously defatted cotton bag and then immersed in 200 mL of hexane in a closed glass at a temperature of ≈25°C. After the sample was stored in a dark place for 24 hrs, the oil was obtained after solvent evaporation using a rotary evaporator (BUCHI Labortechnik AG, Flawil, SG, Switzerland) at 40°C.

2.5. Yield. Oil yield was determined according to Chougui et al. [31], using the following equation:

\[ \text{Oil (g)} = \left( \frac{M_1 - M_2}{M_2} \right) \times 100, \]

where \( M_1 \) is the weight of the empty Eppendorf tube (g), \( M_2 \) is the weight of the Eppendorf tube after evaporation (g), and \( M_3 \) is the weight of the milled seeds (g).

The oil was stored in 2 mL amber Eppendorf tubes at −32°C until analysis.

2.6. Determination of Antioxidant Activity

2.6.1. ABTS Assay. Antiradical capacity by ABTS was measured according to Kuskoski et al. [32]. The radical cation 2,2’azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diazonium salt (ABTS⁺) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate in the dark at room temperature for 16 hrs before being used. The ABTS⁺ solution was diluted with deionized water to an absorbance of 0.70 ± 0.10 at 754 nm. An aliquot of 20 μL of sample was added to 980 μL of the diluted ABTS⁺ solution, and absorbance readings were taken after 7 min incubation at room temperature. The absorbance of the mixture was measured at 754 nm in the microplate reader (Power Wave XS UV-Biotek, software KC Junior, VT, USA), and antioxidant capacity was expressed as mg ascorbic acid equivalent per 100 g of oil (mg AAE/100 g).

2.6.2. DPPH Assay. Antiradical activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH*) radical as described by Morales and Jiménez-Pérez [33]. A methanol-acetate solution (7.4 mg/100 mL) of the stable DPPH* radical was prepared. A sample aliquot of 100 μL was placed into vials and 500 μL of DPPH* solution was added before the mixture was left to sit at room temperature for 1 hr. Finally, absorbance was measured at 520 nm in the microplate reader (Power Wave XS UV-Biotek, software KC Junior, Winooski, VT, USA), and antioxidant activity was expressed as μmol of Trolox equivalents per 100 g of oil (μmol TE/100 g).

2.7. Experimental Design

2.7.1. Optimization. The optimization of the ultrasound extraction conditions was performed using the response surface methodology (RSM) with a central composite rotatable design for two independent extraction variables at five levels. The independent extraction variables (amplitude level: 80–90%; time: 5–15 min) were determined based on preliminary experiments where higher antioxidant activity by ABTS and DPPH was observed. Design consisted in thirteen combinations with five central points replicates (Table 1). Experimental data were subjected to multiple nonlinear regression
where $Y$ is the predicted response, $\beta_0$ is the constant coefficient, $\beta_i$ is the linear coefficient, $\beta_{ij}$ is the quadratic coefficient, and $\beta_{ij}$ is the interaction coefficients. In this model, $X_i$ and $X_j$ are the independent extraction variables, amplitude level (%), and time (min), respectively.

The adequacy of the mathematical model was determined using the coefficient $R^2$. The significance of the model regression coefficients was evaluated using an analysis of variance. Three-dimensional curves from the response surface plots were obtained to interpret the effects of the interaction between independent variables on the response variables. Contour plots were generated to represent the extrapolation and interpret the optimization of the extraction variables, using the Sigma Plot 12.3 graphing software (SYTAT software Inc., Richmond, CA, USA).

### 2.7.2. Treatment Comparison

Comparison between extraction methods (ultrasound-optimized Soxhlet and maceration) was carried out by a one-way analysis of variance (ANOVA). All determinations were performed in triplicate and significant differences between means were determined by Duncan test ($p \leq 0.05$) using the SPSS program (15.0, SPSS Inc., Chicago, IL, USA).

### 2.8. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to examine the morphological alterations caused to the cactus pear seeds before and after the ultrasound extraction. Samples deposited on the silicon wafer were coated with a thin layer of gold (Denton Vacuum Desk V, Moorestown, NJ, USA) applying 20 millitorr and 20 mA during 4 min. Samples were observed in a scanning electronic microscopy (JEOL JSM-6300, Peabody, MA, USA) at 1,000 and 500 amplifications and micrographs were taken to establish the structural comparison between both samples.

### 2.9. Antimicrobial Activity

The green cactus pear seed oil was tested against one Gram-positive bacteria, *Staphylococcus aureus* (ATCC 1654), and two Gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). All microorganisms were obtained from the Mexican Microbial Culture Collection of CINVESTAV of the National Polytechnic Institute (Mexico). For each microorganism, bacterial suspensions were made in a soybean casein digest medium to a concentration of approximately $10^8$ CFU/mL.

To evaluate the antimicrobial activity and the minimum inhibitory concentration (MIC), the disk diffusion method was used. Each bacterial suspension (100 $\mu$L from the $10^8$ CFU/mL) was spread on prepared agar plates (sterile Standard Methods Agar for bacteria). Filter sterile paper discs (6 mm in diameter) were impregnated with 66.67, 50, 33.33, and 16.67 $\mu$L of the undiluted oil and were placed on the inoculated plates. The negative control was hexane, while ampicillin (10 $\mu$g) and streptomycin (10 $\mu$g) were used as positive controls. The plates where incubated at 37 $^\circ$C (Arsa AR-130, Felisa, Jalisco, Mexico) for 24 hrs. The diameters of the inhibition zones were measured in millimeters and the results of MIC were expressed as $\mu$g/$\mu$L.

### 3. Results and Discussion

#### 3.1. Extraction Yield

Table 2 shows the oil yields achieved by the ultrasound treatments. The extraction yield varied from 3.75 to 6% and the maximum yield was obtained at the highest amplitude level of 92%. Oil yield strongly depended on amplitude level probably because at high amplitudes the cavitation effect increases [21] and induces physical changes on the structure of the seed such as disruption of the cell walls, reduction of the particle size, and increase of exposure area. These conditions may facilitate the penetration of the solvent and thus the extraction of oil [26, 28, 34]. The maximum yield was achieved after 10 min of treatment, and longer times (15 min) at high amplitudes (90%) did not increase oil extraction (Table 2). Albeit time is an important variable, after yield reaches a peak, a longer treatment does not maximize extraction; the same was described by Zhang et al. [27] for Korean pine seed, who demonstrated that oil yield increased with time, but when it reached a maximum, yield equilibrated and then decreased gradually. This may be attributed to an initial complete fracture of the cell walls during the first minutes of the cavitation effect [26, 28, 34].

#### 3.2. Antioxidant Activity of Green Cactus Pear Seed Oil Extracted by Ultrasound

Due to the complexity of the oxidation processes, it is advisable to perform more than one method to obtain the antioxidant profile of a sample [35]. In order to determine the antioxidant activity of the green

### Table 1: Experimental design matrix.

<table>
<thead>
<tr>
<th>Number</th>
<th>Pattern</th>
<th>Amplitude level (%)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>00</td>
<td>85</td>
<td>10</td>
</tr>
<tr>
<td>(2)</td>
<td>+−</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>(3)</td>
<td>++</td>
<td>90</td>
<td>15</td>
</tr>
<tr>
<td>(4)</td>
<td>00</td>
<td>85</td>
<td>10</td>
</tr>
<tr>
<td>(5)</td>
<td>A0</td>
<td>92</td>
<td>10</td>
</tr>
<tr>
<td>(6)</td>
<td>−−</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>(7)</td>
<td>00</td>
<td>85</td>
<td>10</td>
</tr>
<tr>
<td>(8)</td>
<td>a0</td>
<td>78</td>
<td>10</td>
</tr>
<tr>
<td>(9)</td>
<td>0A</td>
<td>85</td>
<td>17</td>
</tr>
<tr>
<td>(10)</td>
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<td>80</td>
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</tr>
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<td>00</td>
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</tr>
<tr>
<td>(12)</td>
<td>00</td>
<td>85</td>
<td>10</td>
</tr>
<tr>
<td>(13)</td>
<td>0a</td>
<td>85</td>
<td>3</td>
</tr>
</tbody>
</table>

*Nonrandomized.*

---

**Note:** The table above represents the experimental design matrix with the factors: Pattern, Amplitude level (%), and Time (min). The data is used to analyze the influence of these variables on the predicted response. The model is given by the second-order polynomial equation (2) where $Y$ is the predicted response, $\beta_0$ is the constant coefficient, $\beta_i$ is the linear coefficient, $\beta_{ij}$ is the quadratic coefficient, and $\beta_{ij}$ is the interaction coefficients. In this model, $X_i$ and $X_j$ are the independent extraction variables, amplitude level (%), and time (min), respectively. The adequacy of the mathematical model was determined using the coefficient $R^2$. The significance of the model regression coefficients was evaluated using an analysis of variance.
cactus pear seed oil, two parameters were evaluated: antioxidant and scavenging capacity by ABTS and DPPH, respectively. The results and experimental design are described in Table 2. Antioxidant activity ranged from 55.84 to 68.37 mg AAE/100 g for ABTS and 101.63 to 289.26 μmol TE/100 g for DPPH. A R² value closest to one or at least of 0.80 indicates a good fit of the model [36]. The R² values for ABTS and DPPH were 0.93 and 0.96, respectively (Table 3), indicating that the averages obtained adjusted to the mathematical response surface model.

### 3.3. Effect of Ultrasound Extraction Conditions on the Antioxidant Activity of Cactus Pear Seed Oil

In this study, a second-order polynomial model for predicting the antioxidant activity of green cactus pear seed oil was obtained by multiple linear regression analysis of the experimental data. Table 3 shows the regression coefficients and significant probabilities of the linear, quadratic, and interaction effects of the ultrasound conditions on the seed oil antioxidant activity. Both ABTS and DPPH values were significantly affected by amplitude level in linear term ($\beta_1$) at $p < 0.001$ DPPH and $p < 0.001$ ABTS, respectively, as well as in its quadratic term ($\beta_{11}$) at $p < 0.001$ DPPH and ABTS. The three-dimensional surface plots constructed to observe the effect of ultrasound processing (Figure 1) demonstrated that antioxidant activity was higher when the applied amplitude decreased.

#### 3.4. Optimization of the Ultrasound Extraction Conditions of Cactus Pear Seed Oil

Optimal extraction conditions were selected from the overlapped contour plots in which the effect of amplitude level and time on the antioxidant activity of green cactus pear seed oil was considered.

Figure 2 shows the optimal zone where the highest antioxidant activity by ABTS and DPPH was achieved. The conditions corresponded to amplitude of 78% applied for 10 min. In this zone the values for antioxidant activity were of 66.25 mg AAE/100 g and 289.26 μmol TE/100 g for ABTS and DPPH, respectively.

#### 3.5. Comparison between Methods

**Extraction Yield and Antioxidant Activity.** Figure 3 compares the oil yields obtained by the three extraction methods. The results revealed that the yield obtained using the optimized ultrasound extraction was significantly lower than the obtained with the Soxhlet and maceration procedures. The higher oil yield obtained by Soxhlet could be attributed to the constant and extended contact (4 to 6 hrs) of the sample with the solvent at high temperatures, in addition to the repeated washing cycles [37]. The ultrasound yield was closer to that obtained with the Soxhlet and maceration procedures. The optimized ultrasound extraction was significantly lower than the obtained with the Soxhlet and maceration procedures. The higher oil yield obtained by Soxhlet could be attributed to the constant and extended contact (4 to 6 hrs) of the sample with the solvent at high temperatures, in addition to the repeated washing cycles [37]. The ultrasound yield was closer to that obtained with the Soxhlet and maceration procedures. The higher oil yield obtained by Soxhlet could be attributed to the constant and extended contact (4 to 6 hrs) of the sample with the solvent at high temperatures, in addition to the repeated washing cycles [37]. The ultrasound yield was closer to that obtained with the Soxhlet and maceration procedures. The higher oil yield obtained by Soxhlet could be attributed to the constant and extended contact (4 to 6 hrs) of the sample with the solvent at high temperatures.
oil. Albeit the oil extracted by the Soxhlet method presented higher yield and antioxidant activity, the ultrasound-assisted extraction may be enhanced if multiple extractions are carried out in a time comparable to the required by the Soxhlet procedure (4–6 hrs).

3.6. Antimicrobial Activity. *Escherichia coli* and *Staphylococcus aureus* are distributed in nature (water, soil, and vegetation) and are also part of the human intestinal

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**Figure 1**: Effect of the ultrasound extraction on the antioxidant activity of green cactus pear seed oil. (a) ABTS; (b) DPPH.

**Figure 2**: Optimal ultrasound extraction conditions of green cactus pear seed oil based on the highest antioxidant activity.

**Figure 3**: Cactus pear seed oil yield achieved by different extraction methods. a,b,c Different letters mean significant differences between methods ($p < 0.05$).

**Figure 4**: Antioxidant activity by ABTS and DPPH of cactus pear seed oil extracted by different methods. a,b,c Different letters mean significant differences between methods ($p < 0.05$).
Table 4: Antimicrobial activity of cactus pear seed oil extracted by different methods.

<table>
<thead>
<tr>
<th>Oil*</th>
<th>Diameter of inhibition zone (mm)</th>
<th>(+) control</th>
<th>(-) control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus (Gram-positive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td>9.17 ± 0.29*</td>
<td>18.89 ± 1.54</td>
<td>ND</td>
</tr>
<tr>
<td>Soxhlet</td>
<td>9.50 ± 0.87*</td>
<td>18.78 ± 1.64</td>
<td>ND</td>
</tr>
<tr>
<td>Maceration</td>
<td>9.78 ± 0.69*</td>
<td>18.11 ± 1.17</td>
<td>ND</td>
</tr>
<tr>
<td>Escherichia coli (Gram-negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td>7.78 ± 0.19*</td>
<td>15.56 ± 2.12</td>
<td>ND</td>
</tr>
<tr>
<td>Soxhlet</td>
<td>7.56 ± 0.19*</td>
<td>15.33 ± 2.65</td>
<td>ND</td>
</tr>
<tr>
<td>Maceration</td>
<td>7.56 ± 0.38*</td>
<td>14.00 ± 2.33</td>
<td>ND</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (Gram-negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td>ND</td>
<td>12.56 ± 0.38</td>
<td>ND</td>
</tr>
<tr>
<td>Soxhlet</td>
<td>ND</td>
<td>12.22 ± 0.38</td>
<td>ND</td>
</tr>
<tr>
<td>Maceration</td>
<td>ND</td>
<td>12.78 ± 0.38</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Seed oil: 66.67 μL; (+) control: ampicillin (disc 10 μg; Staphylococcus aureus and Escherichia coli) and streptomycin (disc 10 μg; Pseudomonas aeruginosa); (-) control: hexane; ND: not detected; ±: standard deviation; * Same superscripts indicates that there is no significant difference (p > 0.05).

Microbiota [38]. Pseudomonas aeruginosa, besides being present in the intestinal microbiota [39], is a bacteria found in the soil, fertilizers, and water used for food production [40] and thus it can contaminate fresh or processed food, which is an indicator of inadequate sanitation or improper handling during food production [41]. Table 4 summarizes the antimicrobial activity of green cactus pear seed oil. Extraction method did not have a significant effect on the antimicrobial activity against Staphylococcus aureus and Escherichia coli, but the effect was lower than the positive controls. These results may be explained by the similar seed oil concentration and combined action of compounds on the structure of microbial cells [42, 43], despite the extraction method. Seed oil did not exhibit antimicrobial activity against Pseudomonas aeruginosa, probably due to the oil chemical composition, the type of microorganism, and the own characteristics of the bacteria [42–44]. The antimicrobial activity of oils is generally more effective against Gram-positive bacteria in comparison to Gram-negative bacteria, which are more resistant mainly because their outer membrane is less permeable [42, 43, 45, 46]. The results suggest that Pseudomonas aeruginosa was more resistant than Escherichia coli, probably due to the lipopolysaccharides present in the outer membrane that restrict the diffusion of compounds making it less permeable [45]. The resistance can also be caused by systems of exclusion pumps that eject antimicrobial compounds from the inside of the bacteria before they can cause damage [39, 44].

3.7. Effect of Ultrasound on the Physical Structure. Scanning electron micrographs of the green cactus pear seeds powder before and after the ultrasound treatment at magnifications factors of 1000x and 500x are shown in Figure 5. Before the ultrasound treatment and despite the previous milling process, in the control sample it was possible to identify intact structures of the seeds cell as well as some starch granules (Figure 5(a)). After the ultrasound treatment, the cell structural damage and the variations in the shape and size of the particles were observed (Figure 5(b)). For instance, starch granules were not observed because sonication fragmented these particles while cavitation phenomenon disrupted the cell structures of the seeds [47, 48].

4. Conclusions

This study demonstrated that response surface methodology and a second-order polynomial model were effective tools to determine the optimum processing conditions of ultrasound-assisted extraction based on the maximum values of antioxidant activity. The results demonstrated that cactus pear seed oil has good antioxidant and antimicrobial properties. Ultrasound-assisted extraction was comparable to maceration but a single ultrasound process yielded less oil and lower antioxidant activity than solvent extraction (Soxhlet). Ultrasound can be considered an alternative technology for the extraction of seed oil but further research is required to determine the uses of the seed oil and the technology within the food industry and the potential of several ultrasound cycles at the optimized conditions.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was financially supported by Programa Integral de Fortalecimiento Institucional (PIFI 2014-2015). The authors acknowledge Mexican Association CoMeNTuna (Hidalgo, Mexico) for providing the plant materials. Maria de los Angeles Ortega-Ortega participated in this research and she
received her Bachelor’s degree in nutrition, in the Universidad Autónoma del Estado de Hidalgo, México (Act no. 1287/2016).

References


Research Article

Antioxidant and Antimicrobial Properties of Cactus Pear (Opuntia) Seed Oils

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Received 23 January 2017; Accepted 5 April 2017; Published 26 April 2017

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Seed oils from two Mexican varieties of cactus pear (green: Opuntia albicarpa and red: Opuntia ficus indica) were extracted with different solvents (hexane, ethanol, and ethyl acetate) to evaluate their antioxidant activity. The seed oil with higher antioxidant activity was selected to evaluate antimicrobial activity. The fatty acid profile was analyzed by gas chromatography-mass spectrometry (GC-MS). Oil from green cactus pear seeds obtained with ethanol and ethyl acetate exhibited higher antioxidant activity (p < 0.05) of 323 and 316 μmol TE/20mg (p < 0.05), respectively, compared to red cactus pear seed oil (≈274 and 247 μmol TE/20mg with ethyl acetate and ethanol, resp.). The oil obtained with ethanol and higher antioxidant activity was used to determine the antimicrobial activity. Both cactus pear oils produced a microbial inhibition zone in most of the microorganisms evaluated, particularly Saccharomyces cerevisiae which had similar diameter (38–40mm). The oil fatty acids profiles of both varieties were similar and exhibited a high content of linoleic acid, while two fatty acids (linolenic and behenic) found in red cactus pear were not observed in the green variety.

1. Introduction

A relatively untapped source of lipid and protein raw material is the by-product of fruit-processing plants. Millions of pounds of fruit seeds are discarded yearly causing disposal problems, while proper utilization of these waste products could lead to an important new source of oil and meal [1]. Seeds of fruits collect at least some cytoplasmic lipid bodies as major storage reserve for lipid accumulation [2]. Fruit seeds oils are of great interest because they are edible oils with high degree of unsaturation, antioxidant radical scavenging properties [3–8], and a broad spectrum of antimicrobial activity [9–15]. Therefore, the oil from plants can be potentially used by the food industry for the manufacturing of “natural” or “green” safe foods [16] and extend shelf-life [17, 18].

The oil from cactus pear seed has been found to have an appreciable amount of oil with high levels of unsaturated fatty acids [19], with antioxidant [20, 21] and antimicrobial activity [22], as well as cardioprotective, antithrombotic, anti-inflammatory, antiarrhythmic, hypolipidemic, and antihyperglycemic effect [23, 24]. These properties are of interest for the pharmaceutical and food industry. However, the concentration and effectiveness of these oils may vary among cultivars or varieties, crop environmental factors (e.g., light, temperature, and type of soil nutrients), or methods and solvents used for their extraction. Therefore, the purpose of this research was to determine the antioxidant and antimicrobial activity, and fatty acid profile of the oil obtained from two Mexican varieties of cactus pear (Opuntia albicarpa and Opuntia ficus indica) seeds extracted with different solvents.
2. Materials and Methods

2.1. Plant Material. Two Mexican varieties of cactus pear (Opuntia albicarpa and Opuntia ficus indica) fruit, green (cv. Reyna) and red (cv. Rojo Pelón), respectively, were provided by the Mexican association CoMeNTuna (Consejo Mexicano del Nopal y la Tuna, A.C.; Actopan, Hidalgo, Mexico). Fruits free of external injuries were selected, washed, and manually peeled. Cactus pear seeds were obtained after juice was extracted stirring the pulp with an industrial blender (38BL52 BBC10, Waring Comercial®, USA) and passing it through a conventional strainer. The seeds retained were washed in the strainer with water until pulp residues were removed.

2.2. Powder Seed and Oil Extraction. Green cactus pear seeds (GCPS) and red cactus pear seeds (RCPS) were sun-dried and then grounded (Cyclotec 1093, Tecator Sweden) to a 1mm diameter mesh and stored at \(-23^\circ C\) until further analysis. The seed oil was extracted as follows: 25 g of powdered seeds was mixed with 500 mL of solvents with varying polarities (hexane, ethanol, and ethyl acetate) and the obtained residue was reextracted until extraction solvents become colourless. All the extracts were filtered through filtration paper Whatman number 1 and the filtered extracts were collected for further drying and removal of the remaining solvents at 50\(^\circ C\) using a rotary evaporator (BUCHI, R-200, Switzerland). All extracts were placed in plastic bottles and then stored at \(-20^\circ C\) until used. The oils obtained were used to further analysis.

2.3. Free Radical Scavenging Assay. The free radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH\(^\bullet\)) radical as described by Morales and Jiménez-Pérez [25]. A volume of 500 \(\mu L\) of ethanolic DPPH\(^\bullet\) solution (7.4 mg/100 mL) was added to a sample aliquot of 100 \(\mu L\) placed in vials. The mixture was left to sit at room temperature for 1 h and then was vortexed and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 520 nm in a microplate reader (PowerWave XSUV-Biotek, USA), and \(\mu L\) of Trolox equivalents per 20 milligram (\(\mu mol\) TE/20 mg) of sample was obtained. Oil samples with best antioxidant capacity obtained from the different solvents were used for the antimicrobial analysis.

2.4. Antimicrobial Activity. Eight standard freeze-dried cultures of bacteria, Candida albicans (ATCC 10231), Escherichia coli O58:H21 (ATCC 10536), Escherichia coli O157:H7 (CCUG 44857), Staphylococcus aureus (ATCC 13565), Listeria monocytogenes (CCUG 15526), Pseudomonas aeruginosa (ATCC 15442), Saccharomyces cerevisiae (CECT 1942), and Salmonella Typhi (CCUG 29478) were obtained in thermosealed vials from the Spanish Type Culture Collection (Autonomous University of Barcelona, Barcelona, Spain). Freeze-dried cultures were rehydrated in tryptone soy broth at \(37^\circ C\) for 18 h and then were used to inoculate tryptone soy agar and malt extract agar plates; all microorganisms were incubated at \(37^\circ C\) except Candida albicans and Saccharomyces cerevisiae which were incubated at \(25^\circ C\). Individual colonies were maintained on specific agar slants, stored at 4\(^\circ C\), and subcultured every 15 days.

Disc Diffusion Assay. Antimicrobial activity of oil extracted from GCPS and RCPS was carried out using the disc diffusion method [26]. Petri plates were filled with \(\sim 20\) mL of sterile tryptone soy agar for bacteria and malt extract agar for fungi. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 minutes. Serial dilutions (10−50 \(\mu g/mL\)) of the seed oil from a stock solution (1 mg/mL) were prepared in 20% DMSO and \(10\) \(\mu L\) loaded onto the sterile blank discs (BBL™ Sensi-Disc™) of 6 millimeters of diameter. On the media surface the loaded disks were placed and left for 30 minutes at room temperature to allow compound diffusion. The seed oil was serially diluted in Mueller–Hinton broth medium and duplicate tubes of each dilution (10−100 \(\mu g/mL\)) were inoculated with 5 \(\times\) 10\(^6\) cells of the test bacteria strain and cultures. The antibiotic agents Sensi-Disc streptomycin, ampicillin, and sulfamethoxazole/thrimethoprim (BBL Sensi-Disc) were used as positive controls at the same concentration level. After plates were incubated at \(37^\circ C\) for 24 h, the diameters of the inhibition zones were recorded in millimeters. Three independent repetitions were performed and tests were made in triplicate.

2.5. GC-MS Analysis. The GC-MS analysis was performed with a GC-MS HP-5890 (Hewlett-Packard Company, Palo Alto, California, USA) equipped with a Flame Ionization Detector (FID), and a ZB-WAX fused silica capillary column (60 m \(\times\) 0.25 mm i.d. \(\times\) 0.25 mm film thickness) packed with 5% phenylmethyloxysiloxane (Phenomenex, Torrance, CA). To obtain the methyl esters, the cactus pear seed oils were saponified and derivatized using KOH IN (IUPAC, 1969). Changes in the fatty acids of the oils samples were compared against a standard mixture of 37 components of fatty acids methyl esters (FAMEs) (Food Industry FAMEs Mix, Restek) comprised by methyl esters with chains C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1n9c, C18:1n9t, C18:2n6c, C18:2n6t, C18:3n6, C18:3n3, C20:0, C20:1n9, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C21:0, C22:0, C22:1n9, C22:2, C22:6n3, C23:0, C24:0, and C24:1n9. The sample volume injected was of 2 \(\mu L\) (split ratio 20 : 2) at an injector and detector temperatures of 225 and 225\(^\circ C\), respectively. N2 was used as carrier gas at a flow rate of 1.2 mL/min\(^{−1}\). Fatty acids were calculated as percentage of total FAMEs.

2.6. Statistical Analysis. All values were obtained by triplicate and expressed as means \(\pm\) standard deviations (SD). Data were analyzed using the SPSS V15 software (SPSS Institute Inc., Cary, NC). An ANOVA was carried out to determine differences between oils extracted as well as its antimicrobial activity that were significant at the 5% level of probability and a Tukey test was used for comparison of data.

3. Results and Discussion

3.1. Yield Comparison between Extraction Solvents. Hexane, ethanol, and ethyl acetate were used to extract the oil from
cactus pear seeds. The extraction yields are compared in Figure 1, which shows that the higher amount of oil (%) was obtained from the green cultivar and that yield depended on the solvent used. Oil extraction with hexane was higher for both fruit varieties (11.83% for GCPS and 6.89% for RCPS), followed by ethanol, which reached the same yield as ethyl acetate for GCPS (≈10%). Ethyl acetate was the least effective solvent for RCPS. The extraction yields were similar to those reported (≈7 to 11%) for several varieties of *Opuntia ficus indica* [27–29]. This extraction yield will vary depending on several factors as fruit variety, harvest period, maturation, geographic region, percentage of oil in the seed, and chemical compounds found in the source and by the extraction method [30]. Researchers have determined that solvent extraction combined with other methods could increase oily yield, as high pressure or supercritical fluid combined with solvent reached a yield of 9.33% from tobacco seeds (*Nicotiana tabacum* L.) while sonication and Soxhlet reached a 7.75% and 13.72%, respectively [31].

### 3.2. Free Radical Scavenging Activity

Solvent extraction is usually used for isolation of antioxidants; the extraction depends on the solvent selected based on the different antioxidant compounds with varying polarity [32, 33]. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [34, 35]. The DPPH assay has also been used to predict the oxidative stability of edible oils [36, 37]. The antioxidant activity determined by DPPH of the oil extracted from RCPS and GCPS is shown in Figure 2. Oil from the GCPS extracted with ethanol and ethyl acetate exhibited the higher antioxidant activity (p < 0.05) of 323 and 316 μmol TE/20 mg extract, respectively, followed by RCPS oil extracted with ethyl acetate (274 μmol TE/20 mg extract) and ethanol (247 μmol TE/20 mg extract). These results demonstrate that the extraction solvent had a significant effect on the free radical scavenging capacity of the oil, where the hexane had the lower values. In our study, the green variety exhibited a higher antioxidant activity regardless of solvent. Different results may depend mainly on the content and concentration of bioactive compounds in the oil, but other factors such as solvent polarity, solubility of the extracts in different testing systems, stereoselectivity of the radicals [38], and strong synergism between fatty acids [6] may affect antioxidant activities. Other studies have also reported diverse antioxidant activity among oils from different *Opuntia varieties* [20, 39, 40].

#### 3.3. Antibacterial and Antifungal Activity

The most recommended way to prevent or inhibit microbial growth in foods is the use of food preservatives. Essential oils are secondary metabolites of plants that have wide applications in the food flavoring and preservative industry [41]. Six different bacteria and two fungi species were used to screen the antimicrobial potential of the oils extracted from the two varieties of cactus pear seeds. Oil extracted with ethanol exhibited the highest antioxidant activity and therefore it was used to evaluate the antibacterial and antifungal activity. Figure 3 shows the results from the microbial assay where most microorganisms showed an inhibition zone when exposed to GCPS and RCPS oils, except *Salmonella Typhi* and *Escherichia coli* O157:H7 (image not shown). From these two microorganisms, the first showed an inhibition zone in the presence of antibiotic agents streptomycin (S), ampicillin (AMP), and sulfamethoxazole/trimethoprim (STX) in diameters of 14.6, 11.3 and 27.3 mm, respectively (Table 1), while *Escherichia coli* O157:H7 was only inhibited by SXT (25.3 mm), which agrees with other reports of multiantibiotic resistance of *E. coli* O157:H7 due to the presence of the gene cluster AMR-SSuT [42] and production of beta-lactamase [43]. On the other hand, *Saccharomyces cerevisiae* was highly inhibited (38–40 mm) by the extracted oils but grew in presence of the antimicrobial agents (Figure 3). Similar results were observed for *Candida albicans*, although inhibition zones were smaller and similar for both oils. These observations demonstrate that certain compounds in the cactus pear seed oil have antimicrobial activity. Other researchers also reported similar observations for cactus pear seeds.
Table 1: Diameters of growth inhibition zones (mm) in the presence of oil extracted from cactus pear seeds and conventional antimicrobials.

<table>
<thead>
<tr>
<th>Microbial cultures</th>
<th>Extract</th>
<th>Antimicrobial agent</th>
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<tbody>
<tr>
<td></td>
<td>GCPS</td>
<td>RCPS</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae (CECT1942)</td>
<td>38.3 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Escherichia coli O58:H21 (ATCC 10536)</td>
<td>11.9 ± 0.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.4 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Escherichia coli O157:H7 (CCUG 44857)</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Staphylococcus aureus (ATCC 13565)</td>
<td>12.1 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.1 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Listeria monocytogenes (CCUG15526)</td>
<td>13.3 ± 1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (ATCC15442)</td>
<td>16.4 ± 2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.1 ± 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salmonella typhi (CCUG29478)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Candida albicans (ATCC 10231)</td>
<td>11.1 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>-<sup>d</sup> Different letters in the same row indicate significant differences.

A Inhibition zone diameters for oil and reference antibiotics are means ± SE of three replicas. GCPS: green cactus pear seed oil extract, RCPS: red cactus pear seed oil extract, S: streptomycin (10 µg/disc), AMP: ampicillin (10 µg/disc), and SXT: sulfamethoxazole/trimethoprim (10 µg/disc). ND: not detected activity.

Figure 3: Antimicrobial activity of oil extracted from green cactus pear (G); oil extracted from red cactus pear seeds (R); streptomycin (S); ampicillin (AMP); and sulfamethoxazole/trimethoprim (SXT). Candida albicans (ATCC 10231) (a); Escherichia coli O58:H21 (ATCC 10536) (b); Staphylococcus aureus (ATCC 13565) (c); Listeria monocytogenes (CCUG15526) (d); Pseudomonas aeruginosa (ATCC15442) (e); Saccharomyces cerevisiae (CECT1942) (f).

In the present study, the antimicrobial activity of cactus pear seed oil was more effective against fungi compared to bacteria cultures. These interesting results suggest that there is a link between the oil chemical contents and the antibacterial activity than the major component mixed, so that minor components are critical for the activity and exert a synergistic effect [16, 48, 49].
Table 2: Percentages of FAMEs in crude cactus pear seed oil extracts.

<table>
<thead>
<tr>
<th>FAMEs</th>
<th>Green cactus pear seed oil extract</th>
<th>Red cactus pear seed oil extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.078 ± 0.00</td>
<td>0.066 ± 0.01</td>
</tr>
<tr>
<td>C16:0</td>
<td>12.327 ± 0.09</td>
<td>12.887 ± 0.02</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.429 ± 0.02</td>
<td>0.570 ± 0.01</td>
</tr>
<tr>
<td>C16:2</td>
<td>0.073 ± 0.00</td>
<td>0.540 ± 0.00</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.060 ± 0.01</td>
<td>0.075 ± 0.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.436 ± 0.01</td>
<td>3.389 ± 0.07</td>
</tr>
<tr>
<td>C18:1</td>
<td>16.215 ± 0.03</td>
<td>17.061 ± 0.01</td>
</tr>
<tr>
<td>C18:2</td>
<td>67.448 ± 0.08</td>
<td>65.407 ± 0.01</td>
</tr>
<tr>
<td>C18:3</td>
<td>Ni</td>
<td>0.372 ± 0.01</td>
</tr>
<tr>
<td>C22:0</td>
<td>Ni</td>
<td>0.160 ± 0.01</td>
</tr>
</tbody>
</table>

Means of 3 replicates ± SE. Ni: not identified.

Figure 4: Chromatograms of FAMEs of cactus pear seed oil extract. (a) Green cactus pear seed oil extract; (b) red cactus pear seed oil extract. In both oils extracts were identified: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1, cis-9), hexadecadienoic (C16:2, cis-9, 12), margaric (C17:0), stearic (C18:0), oleic (C18:1, cis-9), linoleic (C18:2, cis-9, 12), except linolenic (C18:3, cis-6, 9, 12), and behenic (C22:0) fatty acids that were identified only in red cactus pear seed.

3.4. Fatty Acid Profile. FAMEs chromatograms and percentages are shown in Figure 4 and Table 2. Cactus pear seed oils contained saturated and unsaturated fatty acids, the linoleic fatty acid being the predominant (67.4% and 65.4% in GCPS and RCPS oils, resp.). Minimal amounts of myristic (C14:0), palmitoleic (C16:1), hexadecadienoic (C16:2), and margaric (C17:0) fatty acids in both oils were also identified. The fatty acids profiles of the two cactus pear varieties were similar; however the GCPS had a slightly higher content of the linoleic acid (C18:2) while the fatty acids linolenic (C18:3) and behenic (C22:0) were in minimal amounts only in the RCPS.

Different studies have established that factors as cultivar type, temperature, and harvest time have a strong influence in parameter as pH, Brix, vitamin C, sugars, and fat content [52, 53]. Oumato et al. [52] found differences in linoleic fatty acid (C18:2) content among cactus pear cultivars. In other study, the oleic acid (C18:1) content was significantly influenced by the cultivar and location interaction [53], providing unique characteristics to the oil.

In comparison with other plants oils, the linoleic acid (C18:2) content of the cactus pear fruit was similar to the levels reported for sunflower oil (62%) [54] and higher than wheat germ oil (55.05%) [55] and soybean oil (52.70%) [56]. The contents of other FAMEs in cactus pear varieties such as palmitic (C16:0), oleic (C18:1), and stearic (C18:0) were similar to those reported for Castilla blackberry (Rubus glaucus Benth) with 11.24%, passion fruit (Passiflora edulis) with 15.47% [57], and grape (Vitis vinifera) with 3.5% [58].

Other researchers have reported similar fatty acids profile to our findings for different plant materials and have analyzed the antimicrobial effectiveness against different microorganisms. For instance, fatty acids found in Allium cepa were found to effectively inhibit Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, and Klebsiella pneumoniae [59]. Oil extracted from Swietenia Macrophylla king seed oil inhibited growth of S. aureus,
S. Typhimurium, and P. aeruginosa [60]. These studies demonstrate that seed oil can inhibit fungi and bacteria, but their efficacy would depend on their concentration levels and specific pathogen [15].

4. Conclusions

Oil yield from the green cactus pear was higher in comparison to the red cultivar and was also influenced by the solvent used. Hexane exhibited high extraction yield while oils extracted with ethanol had the better antioxidant activity. The results demonstrated that oil extracts from both varieties have a noticeable antimicrobial activity against gram-positive and gram-negative bacteria comparable to antimicrobial compounds such as ampicillin, streptomycin, and sulfamethoxazole/trimethoprim. This research provides further incentives to develop additives for the food, cosmetic, and pharmaceutical sectors seeking natural compounds with antimicrobial activity. Further studies are needed to determine the specific component responsible for the antimicrobial activity in cactus pear seeds oil and determine the optimum levels of oil extract and the antimicrobial effectiveness in the food matrix.

Additional Points

Practical Application. Our results suggest that the oils extracted from cactus pear seeds have the potential to be used as a natural antioxidant and antimicrobial agents by the food, cosmetic, and pharmaceutical sectors.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

Acknowledgments

This study was possible thanks to the financial support from the Programa Integral de Fortalecimiento Institucional (PIFI 2013–2015), Mexico. This research project was partially supported by the Food Hygiene Department, Faculty of Veterinary from the Autonomous University of Barcelona, Spain. The authors acknowledge the Mexican association CoMeNTuna (Consejo Mexicano del Nopal y la Tuna, Hidalgo, Mexico) for providing the plant materials.

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