Comprehensive REVIEWS in Food Science and Food Safety

Natural Preservatives for Extending the Shelf-Life of Seafood: A Revisit

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Abstract: Consumer demand for minimally processed seafood that retains its sensory and nutritional properties after handling and storage is increasing. Nevertheless, quality loss in seafood occurs immediately after death, during processing and storage, and is associated with enzymatic, microbiological, and chemical reactions. To maintain the quality, several synthetic additives (preservatives) are promising for preventing the changes in texture and color, development of unpleasant flavor and rancid odor, and loss of nutrients of seafood during storage at low temperature. However, the use of these preservatives has been linked to potential health hazards. In this regard, natural preservatives with excellent antioxidant and antimicrobial properties have been extensively searched and implemented as safe alternatives in seafood processing, with the sole purpose of extending shelf-life. Natural preservatives commonly used include plants extracts, chitosan and chitooligosaccharide, bacteriocins, bioactive peptides, and essential oils, among others. This review provides updated information about the production, mode of action, applications, and limitations of these natural preservatives in seafood preservation.

Keywords: antimicrobial, antioxidant, natural preservative, shelf-life, seafood

Introduction

Seafood, including various species of fish, crustaceans, mollusks, and echinoderms, are excellent sources of protein, fat, vitamins, and minerals; and are popular due to their delicacy with high nutritive value. However, the shelf-life of seafood is limited because of the high contents of various nutrients, neutral pH, and high moisture content (Viji, Venkateshwarlu, Ravishankar, & Gopal, 2017). The rapid microbial and biochemical reactions that occur in seafood immediately after death lead to changes in sensory and nutritional properties that reduce the shelf-life (Olatunde & Benjakul, 2018). Generally, seafood is abundant in polyunsaturated fatty acids (PUFAs), which make it more prone to lipid oxidation. Formation of unpalatable odor and flavor, loss of nutrition, production of unhealthy molecules, and color changes are mainly the consequences of lipid oxidation in seafood (Secci & Parisi, 2016).

Microbiological, chemical, and physical changes contribute to the complexity of seafood spoilage. The initial loss of fish freshness is attributed to indigenous enzymes and chemical reactions, whereas complete spoilage in fish is a function of microbial metabolic activities (Sriket, 2014). The distance between the harvesting or capturing ground and processing facilities, storage temperature, and processing methods are essential in determining the quality and deterioration of seafood. Extrinsic and intrinsic factors and capturing methods can positively or negatively impact the quality and shelf-life of seafood (DeWitt & Oliveira, 2016). During the processing, distribution, and storage of seafood, hazard analysis of critical control point, good hygienic practices, and good manufacturing practices are crucial for controlling the spoilage (Li et al., 2012). Extension of the shelf-life of food products, using diverse preservation techniques and nonthermal technologies has gained an increasing interest because of high demand for fresh chilled foods, especially prime-quality seafood (Olatunde & Benjakul, 2018; Sallam, 2007)

Prevention of nutritional and sensory losses caused by microbiological, enzymatic, or chemical changes, and shelf-life extension of food are usually achieved by chemical preservatives, such as sodium benzoates, sodium nitrite, and sulfur dioxide. Nonetheless, accumulation of these synthetic preservatives in tissues can be detrimental to health (Özdemir, Turhan, & Arıkoğlu, 2012). Treatment with salt is one of the common and oldest natural preservative methods used widely for shelf-life extension of seafood because of its low cost, as well as simplicity (Martínez-Alvarez & Gómez-Guillén, 2013). The effectiveness of salt as a preservative is solely due to its ability to reduce the water activity in the seafood muscle, thereby inhibiting the growth of bacteria and enzymatic activity. Halotolerant and halophilic bacteria grow in salt-preserved seafood, by using energy to exclude salt from their cells, thus avoiding protein aggregation (salting-out) in their cytoplasm (Pikuta, Hoover, & Tang, 2007). Uncontrolled growth of these organisms can also lead to spoilage by fermentation. Nevertheless, salting of seafood can affect the taste and provides a high sodium content in products (Ormanci & Colakoglu, 2015). Other natural preservatives, such as plant extracts, essential oils (EOs), bacteriocins, chitosan, and bioactive peptides have been reported to effectively

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replace synthetic preservatives or chemicals because of their excellent antimicrobial and antioxidant properties (Sultanbawa, 2011), especially for preventing deterioration caused by microorganisms, lipid oxidation, or both (Erkan, Doğruyol, Günlü, & Genç, 2014). This review aims to provide updated information about the production, antimicrobial and antioxidant mechanisms, application, and limitations of some commonly used natural preservatives in seafood. Seafood processors and consumers can gain benefit in selecting the potential natural preservatives that can contribute to ensuring safe and high-quality seafood for consumption.

Seafood Spoilage

Any change in the initial condition of seafood that results in an unpalatable odor, taste, appearance, and texture is referred to as spoilage. This change can be attributed to enzymatic, chemical, or microbial activities in the seafood (Ghaly, Dave, Budge, & Brooks, 2010). Adebowale, Dongo, Jayeola, and Orisajo (2008) reported that rigor mortis, which is a biochemical change in fish muscle that occurs immediately after death, led to the loss of muscle flexibility. Activities of indigenous proteases and lipases, spoilage organisms, and lipid oxidation have been reported to be responsible for spoilage or deterioration during post-rigor mortis storage (Ghaly et al., 2010; Grant, Corkum, & Morry, 2003). Chemical, microbial, and enzymatic spoilage in seafood can be controlled by pretreatment, preservatives, and packaging in which the sensory and nutritional properties of the product can be maintained.

Microbial spoilage in seafood

Seafood is highly vulnerable to invasion by opportunistic and pathogenic microorganisms. Habitat, which is a microbe-rich environment, mostly determines the microbial load of seafood (Ghaly et al., 2010; Kuley et al., 2017). Generally, spoilage in seafood is mainly caused by the growth and metabolism of microorganisms associated with the production of biogenic amines, alcohols, histamine, putrescine, sulfides, organic acids, aldehydes, and ketones (Kuley et al., 2017). Psychrophilic bacteria are the main group of microorganisms responsible for spoilage in chilled or refrigerated seafood. Sivertsvik, Jeksrud, and Rosnes (2002) identified aerobic or facultative anaerobic psychrotrophic Gram-negative bacteria, such as *Moraxella*, *Shewanella putrefaciens*, *Acinetobacter*, *Pseudomonas*, *Photobacterium*, *Aeromonas*, *Flavobacterium*, and *Vibrio*, as major spoilage organisms in seafood. Specific spoilage organisms such as *Shewanella*, *Photobacterium phosphoreum*, and *Pseudomonas* are considered the major causes of seafood spoilage (Gram & Dalgaard, 2002).

Gram-negative bacteria are the major contributors to spoilage in seafood. However, continuous processing or extended storage/transportation provides opportunities for Gram-positive bacteria to also dominate and cause spoilage (Al Bulushi, Poole, Barlow, Deeth, & Dykes, 2010). Dalgaard (2000) reported both Gram-negative bacteria (*P. phosphoreum*) and lactic acid bacteria (LAB) as the major spoilage bacteria in fish. Gram-positive bacteria, such as *Micrococcus*, *Corynebacterium*, *Bacillus*, *Staphylococcus*, *Clostridium*, *Streptococcus* (Al Bulushi et al., 2010), and *Brochothrix thermosphacta* (Fall, Leroi, Cardinal, Chevalier, & Pilet, 2010; Lalitha et al., 2005) were also identified as spoilage micro organisms in seafood. It could, therefore, be deduced that both Gram-negative and Gram-positive bacteria are responsible for the spoilage of seafood. However, the sampling location, geographic location, and method of fishing are factors determining the type and number of microorganisms (Ghaly et al., 2010). The low-molecular-weight substances, such as small peptides, carbohydrates, and free amino acids in the tissue, are utilized by microorganisms as an energy source for growth and production of several by-products, including biogenic amines (Masniyom, 2011), histamine (Visciano, Schirone, Tofalo, & Suzzi, 2012), sulfur-containing compounds (Varlet & Fernandez, 2010), and other components.

The enzymatic activity of some other bacteria, such as psychrotolerant Enterobacteria, *Vibrio* spp, *Aeromonas* spp, and *S. putrefaciens* have been reported to reduce trimethylamine oxide (TMAO) in seafood to trimethylamine (TMA), which is responsible for the fishy odor (Arfat, Benjakul, Vongkamjan, Sumpavapol, & Yarnpakdee, 2015; Lidbury, Murrell, & Chen, 2014). TMA production is accompanied by the development of hypoxanthine, which causes a bitter taste in seafood (Tikk et al., 2006). Production of hypoxanthine is induced by indigenous enzymes or, relatively more quickly, by bacteria via decomposition of nucleotides (inosine or inosine monophosphate (Masniyom, 2011; Varlet & Fernandez, 2010; Visciano et al., 2012).

Chemical deterioration in seafood

In general, seafood is rich in lipids, especially fats containing long-chain PUFAs (Venugopal & Gopakumar, 2017). Lipids play a major role in off-flavor and off-odor development and loss in the nutritional value of seafood (Mariutti & Bragagnolo, 2017). Depletion of fat-soluble vitamins and other compounds is also a consequence of lipid oxidation (Kolakawska & Bartosz, 2014; Souza & Bragagnolo, 2014).

Lipid oxidation involves several stages. The mechanisms of lipid oxidation are illustrated in Figure 1. An abstracted labile hydrogen atom from a fatty acyl chain can initiate lipid oxidation, with free radical production. Metals, ions, irradiation, and heat are catalysts for the free-radical formation. Free radicals react expeditiously with oxygen to form the peroxyl radical, which can further abstract a hydrogen atom from another fatty acyl chain, thereby producing a new free radical and hydroperoxide. The new free radical can then continue the chain reaction (Ladikos & Lougovois, 1990; Waraho, McClements, & Decker, 2011).

Lipid oxidation is terminated when there is a build-up of free radicals with the formation of non-radical products (Schneider, 2009). The rate of oxidation is governed by oxygen availability, light, the presence of metals, and moisture, temperature, and degree of unsaturation of the lipid (Maqsood & Benjakul, 2010). Nevertheless, primary products, mainly hydroperoxides, are not stable. Secondary products of lipid oxidation are formed as a result of decomposition of primary products. Thus, both primary (free fatty acids [FFAs], dienes, and peroxides) and secondary (aldehydes, trienes, and carbonyls) products are generated from the lipid oxidation process. Overall, the amounts and types of oxidation products depend on the extent of the oxidation reaction and fatty acid composition (Berton-Carabin, Ropers, & Genot, 2014). Mariutti and Bragagnolo (2017) noted that pre-slaughter activities (physical injuries and stress), post-slaughter activities (cold shortening and tenderization techniques, temperature, and pH), and processing parameters (raw materials quality, processing temperature, size reduction, additives, type of packaging, and distribution and storage conditions), were factors influencing the intensity and rate of oxidation.

Lipid oxidation can be induced by several prooxidants (hemoglobin, myoglobin, and cytochrome c) (Ghaly et al., 2010). Deoxygenated or oxidized hemoglobin, which are prooxidants mostly found in the blood of fish, are responsible for accelerated lipid oxidation (Undeland, Hall, Wendin, Gangby, & Rutgersson,

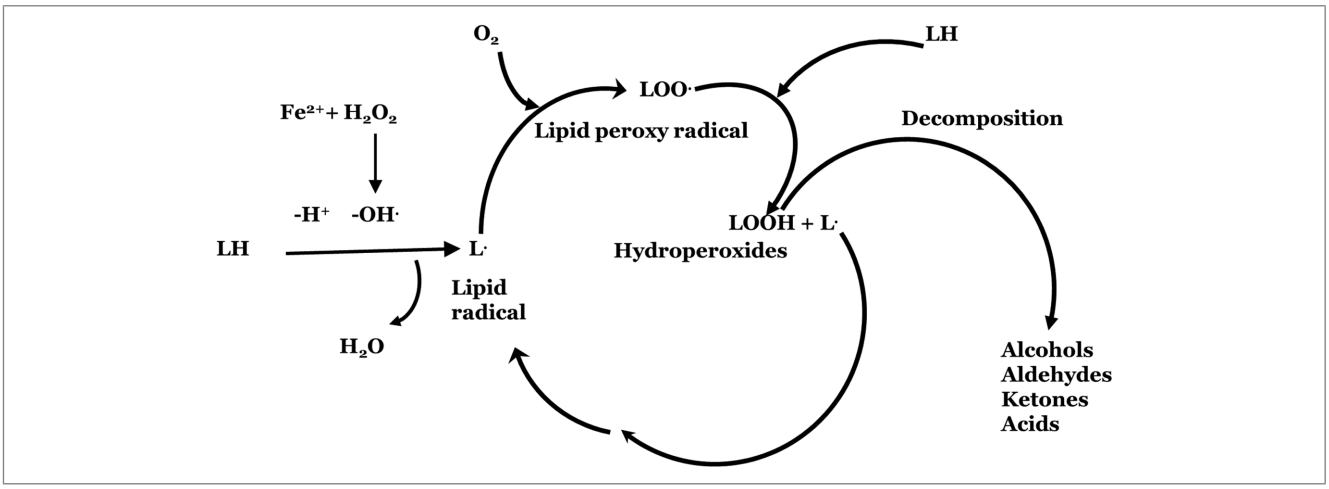

Figure 1–Mechanism of lipid oxidation in seafood. LH stands for fatty acid.

2005). Bleeding could lower lipid oxidation, associated with the decreased prooxidant present in the blood (Maqsood, Benjakul, Abushelaibi, & Alam, 2014; Secci & Parisi, 2016). Apart from the off-flavor development in seafood, loss of functionality, as a result of protein oxidation, occurs when secondary oxidation products react with proteins, amines, and peptides (Estévez & Luna, 2017). Denaturation of myofibrillar and sarcoplasmic proteins is also a result of interaction between those proteins and FFAs formed during hydrolysis of lipids (Edwards, Creamer, & Jameson, 2008).

Enzymatic deterioration in seafood

The process of degrading proteins by indigenous enzymes, known as autolysis, starts immediately after the completion of rigor mortis. This process creates a favorable environment for bacterial growth (Ghaly et al., 2010). Alteration in the sensory properties of seafood can be attributed to proteases and lipases (Engvang & Nielsen, 2001). Even during refrigeration and frozen storage, autolysis occurs in seafood at a very slow rate (FAO, 2005). However, during improper storage, protein is rapidly degraded, via a process mediated by indigenous and microbial proteases. Chymotrypsin, cathepsins, trypsin, lipase, and phospholipase are reportedly found in the hepatopancreas, spleen, and pyloric ceca of seafood, whereas pepsin is located in the stomach (Odedeyi & Fagbenro, 2010). Belly burst, which commonly occurs in fish, is a function of the enzymes in the fish gut, causing rapid protein decomposition. Textural changes (meat toughening) along with the production of formaldehyde, during the storage and processing of seafood, are also results of enzyme activities (Ghaly et al., 2010). Trimethylamine oxide demethylase, found in some fish, induces the formation of formaldehyde by demethylation of TMAO to dimethylamine (DMA) and formaldehyde (Gonçalves & de Oliveira, 2016; Leelapongwattana, Benjakul, Visessanguan, & Howell, 2005). Formaldehyde cross-links proteins via methylene bridging, which makes fish muscle tough and with low water-holding capacity (Immaculate & Jamila, 2018). Melanosis (black pigment formation) in shrimp is also a result of tyrosinase or polyphenol oxidase present in the shrimps (Sae-Leaw, Benjakul, & Simpson, 2017a).

The products of proteolysis (free amino acids and peptides) can serve as the nutrients for microbial growth, leading to spoilage, in conjunction with the formation of biogenic amines (Fraser & Sumar, 1998; Ghaly et al., 2010). Temperature and pH are factors affecting protease activity. Optimum pH values for most proteases are within alkaline and neutral range (Ghaly et al., 2010). The reduction of TMAO to TMA and other basic volatiles by the action and metabolism of the endogenous or microbial enzymes increases the pH of stored seafood (Leelapongwattana et al., 2005; Leelapongwattana, Benjakul, Visessanguan, & Howell, 2008)

Fat in seafood can also be hydrolyzed by lipase or phospholipase (Aryee, Simpson, & Villalonga, 2007; Kaneniwa, Yokoyama, Murata, & Kuwahara, 2004). Hydrolysis of mono-, di-, and triglycerides to glycerol and fatty acids is induced by triacylglycerol acylhydrolases (EC 3.1.1.3) in the presence of water (Fernandes, 2016). FFAs liberated in seafood can readily undergo oxidation, which contributes to the formation of off-odor, particularly fishy odor. The fishy odor is related to aldehydes, mainly polyunsaturated aldehydes (Maqsood et al., 2014). The fishy odor intensity in fish with bleeding was lower than that of unbled fish (Maqsood et al., 2014). Additionally, lipoxygenase located mainly in gill or skin can induce oxidation in stored fish, particularly when the fish is stored for an extended time (Sae-leaw, Benjakul, Gokoglu, & Nalinanon, 2013).

Plant Extracts and Essential Oils

The demand for natural antioxidants and antimicrobials is increasing for seafood and its products (Soto, Falqué, & Domínguez, 2015). For thousands of years, plant extracts have been used for pharmaceutical, medical, phytotherapy, and sanitary purposes, as well as for aromatherapy, perfumery, and cosmetic applications, in addition to the uses in foods and beverages (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Hammer, Carson, & Riley, 1999). Nowadays, plant extracts and EOs are considered natural preservatives or food additives with strong antibacterial, antifungal, and antioxidant activities, used in the food industry for raw and processed food preservation (Benkeblia, 2004; Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007). EOs and their components are used also for their nematocidal activities as reported by Giarratana et al. (2015), Giarratana et al. (2017), Gómez-Mateos Pérez, Navarro Moll, Merino Espinosa, and Valero López (2017), and Trabelsi et al. (2018).

Compounds derived from the secondary metabolism of plant materials, such as leaves, shrubs, fruits, or peel, having one or more phenolic rings, can be regarded as plant polyphenols (Parr & Bolwell, 2000). Maqsood et al. (2014) divided phenolic compounds into two categories, flavonoids and non-flavonoid polyphenols.

The target class is flavonoids, which can be separated, according to the oxidation degree of the heterocyclic ring, into different subclasses: flavonols, flavanols, isoflavones, flavones, flavans, and anthocyanins. Phenolic acids and non-flavonoids are also abundant in plants (Scalbert & Williamson, 2000). Carotenoids, retinoids, tocopherols, ascorbic acid, phenolic acids, terpenoid, flavonoids, and polyphenols are known to be responsible for the antimicrobial and antioxidant activities of plant extracts and EOs (Bakkali et al., 2008; Tongnuanchan & Benjakul, 2014).

Preparation and extraction of plant extracts and essential oils

Plant extracts and EOs, which contain aromatic and aliphatic compounds, can be derived from all organs or some specific tissues of plants, such as petals, leaves, fruits, peels, stems, roots, and xylems (Palazzolo, Laudicina, & Germana, 2013). The quantity and quality of EO and plant extract strongly depend on the nature of the plant, its provenance, soil type, genotype, climate, the age of the plant, extraction medium, and extraction process (Palazzolo et al., 2013; Tongnuanchan & Benjakul, 2014). Production or extraction of EO and plant extract involves two major steps, namely, pre-extraction (preparation) of plant samples and an extraction process (Azwanida, 2015). Drying, grinding, or size reduction of the plant materials and, sometimes, dechlorophyllization, depending on the plant material, are the major processes in the pre-extraction stage (Azwanida, 2015; Chotphruethipong, Benjakul, & Kijroongrojana, 2017). Olatunde, Benjakul, and Kitiya (2018) demonstrated that solvents have a profound role in dechlorophyllization of guava leaf, in which the ethanolic extract from chloroform-dechlorophyllized leaf powder showed the highest antioxidant efficacy. However, the extract of leaves dechlorophyllized with acetone had very low yield.

The separation of soluble phenolic compounds from insoluble marc (residue) using selective solvents through standard procedures is the second stage of production, known as the extraction process (Handa, Khanuja, Longo, & Rakesh, 2008). Water, methanol, ethanol, acetone, isopropanol, hexane, dichloromethane, and toluene are the major extraction solvents used (Grodowska & Parczewski, 2010). Maceration, decoction, percolation, infusion, Soxhlet extraction, ultrasound-assisted extraction or sonication extraction, accelerated solvent extraction, supercritical fluid extraction, and microwave-assisted extraction have been used on plants as extraction methods (Azwanida, 2015; Tongnuanchan & Benjakul, 2014). Various extraction procedures and their advantages and limitations for several plants were documented by Azwanida (2015).

Antimicrobial, antioxidant efficacy, and application of plant extracts and essential oils for seafood preservation

Phenolic compounds in plant extracts or EOs can disrupt the cell membrane integrity by interacting with membrane proteins of bacteria or fungi. By increasing the permeability of the cell membrane, these compounds cause the leaching of potassium ions and other cytoplasmic structures and ultimately, cell death (Bajpai, Shukla, & Kang, 2008; Simoes, Bennett, & Rosa, 2009). The cell membrane of bacteria contains phospholipids, which can be partitioned by the insertion of the hydrophobic compounds of the plant extract or EO, thereby rendering the cell membrane permeable and disrupting the cell structure (Jose, Cyriac, Pai, Varghese, & Shantaram, 2014; Sikkema, de Bont, & Poolman, 1994). Cell death is also caused by extensive leaching of critical ions and the disruption of the bacterial cell wall (Ajaiyeoba, Onocha, Nwozo, & Sama, 2003). Gram-negative bacteria possess a thinner cell wall than Gram-positive bacteria, meaning they are more sensitive to inhibition by plant extracts or EOs (Abdollahzadeh, Rezaei, & Hosseini, 2014). Figure 2 summarizes the potential mechanisms by which antimicrobial agents, such as plant extracts or EOs, inhibit bacterial growth.

Plant polyphenols or extracts have been demonstrated to prevent lipid oxidation in seafood rich in PUFAs (Secci & Parisi, 2016). Maqsood et al. (2014) reviewed the role of plant phenolic extracts on the prevention of lipid oxidation in seafood, and they highlighted how differences in the composition of the phenolic compounds are associated with the variation in the efficacy against lipid oxidation. Antioxidants can retard lipid oxidation by inhibiting the formation of free radicals or interrupting their propagation via one or more mechanisms: (1) scavenging species that trigger peroxidation, (2) chelating metal ions so they cannot generate reactive species that initiate oxidation, (3) quenching O_2 and thereby preventing the formation of peroxides, (4) breaking the auto-oxidative chain reaction, and/or (5) reducing the O_2 concentration (Babizhayev, 2016). The ability to interrupt free-radical chain reactions is the major criterion for ascertaining the effectiveness of antioxidants (Maqsood et al., 2014). The phenolic hydroxyl groups are effective hydrogen donors. After interacting with the initial reactive radical species, the polyphenol itself becomes a radical. However, the formation of quinone structures and the resonance-induced delocalization of electrons within the aromatic ring stabilize these radical intermediates (Nawar, 1996). Hence, lipid oxidation in seafood can be prevented by plant polyphenols (Maqsood et al., 2014).

Plant extracts have been employed in seafood to maintain the quality, as well as to extend the shelf-life, by lowering microbial and chemical reactions. Based on the microbial load, TMA, and thiobarbituric acid-reactive substances (TBARS), the shelf-life of sardine fillets treated with 10% cactus fruit-peel extract was extended to 12 days as compared to 7 days for the control (untreated sample) (Besbes, Joffraud, Ben Khemis, Amri, & Sadok, 2016). The addition of 2% grape seed or 2% clove bud extracts delayed lipid oxidation, lowered the changes in lightness (L^*), redness (a^*), salt-soluble protein content, and total sulfhydryl groups in silver carp (*Hypophthalmichthys molitrix)* fillets stored at 4 °C for 18 days, and extended the shelf-life of fillets by 3 days, compared to the control (Shi, Cui, Yin, Luo, & Zhou, 2014). Algal extracts have also been used for the shelf-life extension of seafood. According to Li, Yang, and Li (2017), the increases in total volatile basic nitrogen (TVB-N), TBARS, and *K*-value (amount of adenosine triphosphate and related compounds) were suppressed in Pacific white shrimp treated with algal (*Porphyra yezoensis*) extracts (5 g/L) during refrigerated storage (4 °C). Also, the total viable count (TVC) and polyphenol oxidase activity were significantly lower in treated samples, compared to the control group, and the shelf-life of treated samples was extended to 8 days, better than the 3 days for the untreated counterpart.

For ease of implementation, plant extracts can be incorporated in the ice used for keeping seafood. Melting of the ice will then release the active components from the plant extract, for the preservation of the stored seafood. Bensid, Ucar, Bendeddouche, and Özogul (2014) stored anchovy in ice containing thyme (0.04% w/v), oregano (0.03% w/v), and clove (0.02% w/v) extracts, respectively. Gutted and beheaded anchovy stored in ice containing the individual extracts had a 12-day shelf-life, whereas those kept in traditional ice had a shelf-life of only 5 days.

The effect of bay leaf, thyme, rosemary, black seed, sage, grape seed, flaxseed, and lemon EOs at a final level of 1% (based on fish

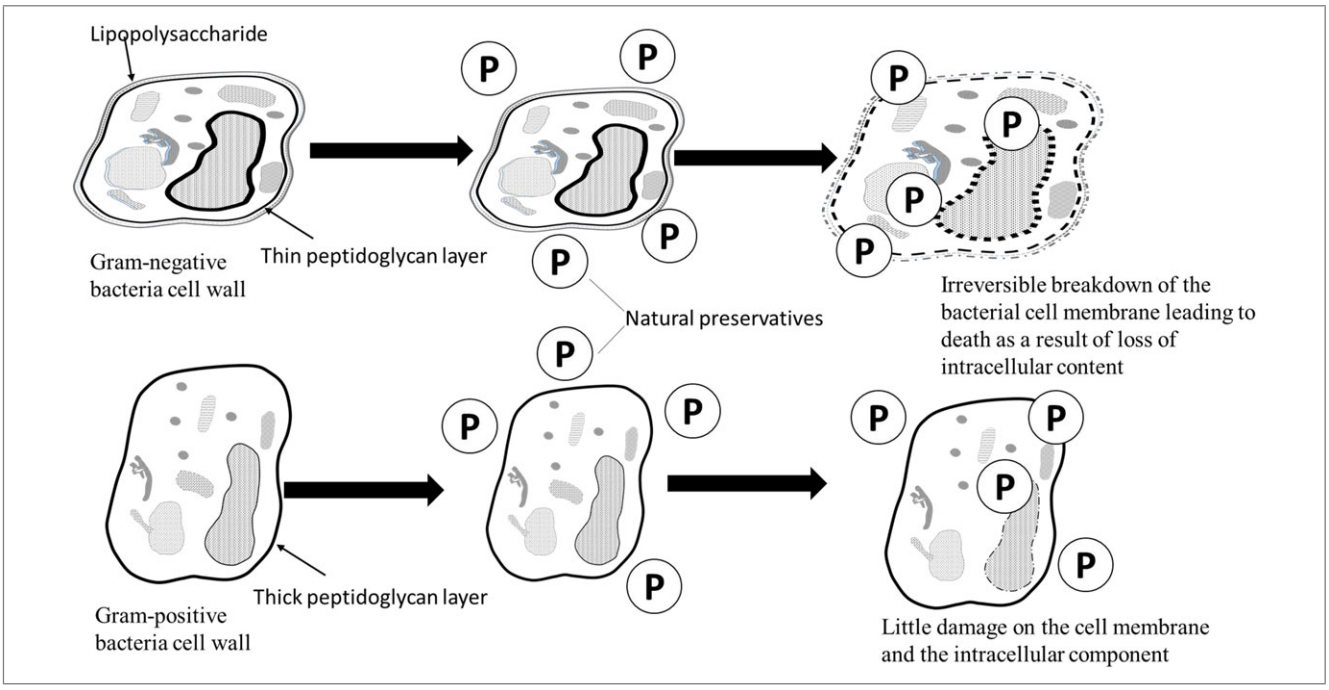

Figure 2–Response of Gram-positive and Gram-negative bacteria to preservatives.

flesh weight, v/w) on the shelf-life of chub mackerel during frozen storage (–20 °C) was studied (Erkan & Bilen, 2010). Analysis of the sensory properties revealed that the shelf-life of frozen chub mackerel treated with bay leaf, grape seed, and flaxseed EOs was extended to 7 months while the remaining treated and untreated samples had a shelf-life of 6 months (Erkan & Bilen, 2010). According to several microbiological (TVC, H_2S-producing bacteria, LAB, *Pseudomonas* counts, *Enterobacteriaceae* counts) and chemical (TVB-N, TMA-N, TBARS) and sensory evaluation, Kostaki, Giatrakou, Savvaidis, and Kontominas (2009) prolonged the shelf-life of seabass at 4 °C, to 17 days after a combined treatment of thyme EO (0.2%) and modified atmosphere packaging (60% CO_2:30% N_2:10% O_2) relative to 6 days for the control sample (packaged in air). Similarly, an independent study recorded a dose-dependent antimicrobial effect of thymol EO (500–1500 ppm) for shrimps stored under modified atmosphere packaging (5% O_2:95% CO_2), effectively prolonging the shelf-life to 14 days compared to 5 days for the nontreated control (Mastromatteo, Danza, Conte, Muratore, & Del Nobile, 2010).

Apart from raw seafood, EOs have been used in finished or processed seafood. Hot smoked rainbow trout was treated with 1% of several EOs, including bay leaf, rosemary, black cumin seed, and lemon, vacuum-packaged, and stored at 2 °C (Erkan, Ulusoy, & Tosun, 2011). The TVC, total psychrotrophic count, LAB count, TMA-N, TVB-N, and TBARS values were lower in the EO-treated samples than the control during storage for 7 weeks. Moreover, the shelf-life of all the EO-treated samples was 6 weeks, except those treated with bay leaf EO, which could be stored up to 7 weeks, whereas the control had a 4-week shelf-life.

Natural active compounds are poorly soluble in water and biologically unstable. Consequently, these active molecules distribute poorly to the target site or cells (Mazandrani, Javadian, & Bahram, 2016). Encapsulation is a common delivery system that entraps active agents within a carrier matrix to improve the bioavailability and stability of the active material (Shoji & Nakashima, 2004). The benefits of encapsulation include (1) reduced reactivity between the natural compounds and environment (light, oxygen, and water); (2) reduced transfer rate of the active compounds to the outside environment and loss due to evaporation; and (3) improved half-life, bioavailability, and handling ability of the compounds (Fang & Bhandari, 2010). Encapsulation of natural compounds will enhance their uniform distribution in the food, when used in very little quantity, and also mask any unpleasant taste associated with the material (Liolios, Gortzi, Lalas, Tsaknis, & Chinou, 2009). Gortzi, Lalas, Chinou, and Tsaknis (2006) improved the stability, antioxidant, and antimicrobial properties of *Thymus* spp. extract by encapsulation in liposomes. Mazandrani et al. (2016) recorded the least lipid oxidation, TVB-N content, and microbial load during storage of silver carp fillets at 4 °C treated with liposomal encapsulated fennel extract (FE) compared with the pure extract treatment and control. The shelf-life of silver carp fillets was extended from 6 days for the control to 15 days when treated with 0.5% encapsulated FE and 12 days after treatment with 0.5% pure extract. In minced kilka fish with added gum Arabic unencapsulated FE (0.3% and 0.5% w/w) and encapsulated FE (0.3% and 0.5% w/w), the chemical (PV and TVB-N) and microbial deterioration (total plate and total psychrotrophic counts) were lower in the encapsulated FE-added minced samples during storage at 4 °C for 15 days (Bagheri, Izadi Amoli, Tabari Shahndasht, & Shahosseini, 2016). Furthermore, the shelf-life was 3, 9, and 12 days for the control, samples added with unencapsulated, and encapsulated FE, respectively. The efficacy in shelf-life extension was dependent on the dose of FE applied.

Another strategy to integrate EOs or plant extracts into food is via their incorporation into films for wrapping (termed active packaging), in which the active components can be gradually released to perform their function in the target food. For example, the biochemical (TVB-N), physical (pH and weight loss), and

Table 1–Essential oil application in shelf-life extension of fish.

Essential oil	Seafood	Antioxidant activity	Antimicrobial activity	References
R(+) limonene (LMN)	Gilthead sea bream (*Sparus aurata*)		R(+) limonene showed a zone of inhibition of 7.9, 8.1, 7.1, 7.4, 17.5, and 7.6 mm against *Pseudomonas fluorescens, P. putida, P. syringae, P. fragi, Shewanella putrefaciens, and S, baltica,* respectively. LMN maintained specific spoilage organisms populations in treated sea bream fillets at a significantly lower level compared with the control samples during the storage. The LMN treated samples had a shelf-life of 15 days compared to 6 days for the control.	Giarratana et al. (2016)
Allyl Isothiocyanate (AITC)	Gilthead sea bream (*Sparus aurata*)		AITC extended the shelf-life of sea bream fillets by maintain a SSO population below 6 log CFU/g even at day 14 as compared to the 6 days for the control.	Muscolino et al. (2016)
Oregano oil	Rainbow trout (*Oncorhynchus mykiss*)	Lipid oxidation as measured by Thiobarbituric acid reactive substance (TBARS) were lowered in oregano treated sample than the control	Total viable bacteria, H2S producing bacteria, LAB, *Pseudomonas* spp., and Enterobacteriaceae were lower in oregano oil treated samples than the control. The shelf-life of treated samples was extended to 21 days as compared to control (6 days).	Frangos, Pyrgotou, Giatrakou, Ntzimani, and Savvaidis (2010)
Allyl isothiocyanate and limonene	Gilthead sea bream fillets		Samples treated with essential oil had a lower SSO count had the control.	Giuffrida et al. (2017)
Aloysia triphylla essential oil (AT)	Silver catfish		AT delayed the onset and resolution of rigor mortis as well as the degradation of IMP into HxR compared to the control in vivo transport. Fillets treated with AT (40 μL/L) had a longer sensory shelf-life than the control.	Daniel et al. (2014)

microbiological quality of flounder (*Paralichthys orbignyanus*) fillets packaged in agar film incorporated with fish protein hydrolysate (FPH) (0.5 g FPH/g agar) were slightly lower than samples packaged with the film containing clove EO (0.5 g EO/g agar), although both films extended the shelf-life of the fillets (da Rocha et al., 2018). In another example, the shelf-life of rainbow trout wrapped using 8% gelatin films with and without laurel EO (1% v/m) was extended to 22 and 20 days, respectively, versus 15 days for the control (without gelatin films), during storage at 4 °C (Alparslan, Baygar, Baygar, Hasanhocaoglu, & Metin, 2014). Also, Thaker, Hanjabam, Gudipati, and Kannuchamy (2017) reported that Indian salmon fillets coated with 10% gelatin solution, 10% gelatin + 30% lime juice + 1.5% chitosan, and 10% gelatin + 30% garlic extract + 1.5% chitosan had a shelf-life of 8, 16, and 16 days, respectively, versus 4 days for the control. In another study, the quality (chemical and microbial indices) of tuna fillets coated with tuna skin gelatin-based edible coating containing 1% macroalgal extracts (*Codium* spp. and *Fucus vesiculosus*) was maintained during storage at 4 °C for 12 days (Vala, Augusto, Horta, Mendes, & Gil, 2017). Lee, Yang, Lee, and Song (2016) prolonged the shelf-life of fatty tuna meat stored at 4 °C for 12 days by wrapping the product in a composite film (1% gelatin + 4% red pepper seed meal protein + oregano EO). A decreased growth of inoculated *Listeria monocytogenes* and *Salmonella typhimurium* in the treated/wrapped samples was noticeable when 0.5% oregano EO was used, compared to the control, and the lipid oxidation of the fish was also decreased. Shrimp coated with edible gelatin coating solution enriched with 2% orange leaf EO improved the sensory (lowered melanosis) and microbiological qualities and increased the shelf-life (14 days) relative to the control (6 days) (Alparslan et al., 2016). Other recent applications of EO in extending the shelf-life of fish are summarized in Table 1.

Limitations of plant extracts and essential oils for seafood preservation

Even at very low concentrations, plant extracts and EOs have been shown to have excellent antimicrobial and antioxidant activities *in vitro*. In the food system, they do not render the remarkable results displayed by many pure compounds. However, some plant extracts, such as rosemary, have been introduced into the market (Sofowora, Ogunbodede, & Onayade, 2013). The application of plant extracts and EOs in food systems, especially seafood, usually requires a higher amount or concentration than synthetic compounds. These levels are not organoleptically acceptable in terms of appearance and color (García-Díez et al., 2016; Holley & Patel, 2005). Even at low concentration, EOs, mostly from herbs and spices, have a strong aroma, which negatively affects the aroma and taste of the treated seafood (Silva-Angulo et al., 2015). Also, chemical variation in plants, due to the age of the plant, ecological and geographical condition, harvesting time, and method of extraction are factors limiting the applications of their extracts as natural food preservatives in seafood (Weerakkody, Caffin, Turner, & Dykes, 2010).

Chitosan and Chitooligosaccharide

After cellulose, chitin is the second most abundant natural polymer, which is derived from crustacean shells (Erkan et al., 2014; Shahidi, Kamil, Jeon, & Kim, 2002). The deacetylation

of chitin yields chitosan [poly-$\beta(1\rightarrow4)$-2-amino-2-deoxy-D-glucopyranose], while the depolymerization of chitosan gives rise to chitooligosaccharide, known as chitosan oligomer or chitooligomers (Benjakul, Visessanguan, Phatchrat, & Tanaka, 2003; Lodhi et al., 2014). The broad application of chitosan and chitooligosaccharides in the food industry is attributed to their nontoxic, degradable, and natural attributes (Alishahi & Aïder, 2012). The presence of an amine, acetylated amine groups, and hydroxyl group in the structure of chitosan and chitooligosaccharide, which can interact with cell receptors, triggers a series of reactions in living organisms. This property is the major factor responsible for their anticancer, antiangiogenic, neuroprotective, immunostimulatory, antidiabetic, antioxidant, and antimicrobial properties (Lodhi et al., 2014). The ability of chitosan and chitooligosaccharide to dissolve in acidic solutions and water widen their applications, as either an additive, coating agent, or film, used for extending the shelf-life of seafood (Yingyuad et al., 2006). As a food packaging material, chitosan has been used for both coating and wrapping materials (Sathivel, Liu, Huang, & Prinyawiwatkul, 2007).

Production of chitosan and chitooligosaccharide

Chitosan is produced from chitin either by alkaline deacetylation, which involves boiling (100 °C) chitin in concentrated alkali for several hours (Alishahi & Aïder, 2012) or by chitin deacetylases (Hembach, Cord-Landwehr, & Moerschbacher, 2017). In nature, chitin exists as a linear structured polysaccharide of *N*-acetyl-D-glucosamine, which has three polymorphic forms, known as α-, β-, and γ-chitin, based on different microfibril orientations (Lodhi et al., 2014; Tharanathan, 2003). Antiparallel α-chitin is considered the most stable polymorphic form in insect chitinous cuticles and crustacean shells (Kameda, Miyazawa, Ono, and Yoshida (2005). Two major steps in the production of chitin from shrimp heads are demineralization (HCl at 1.25 N) and deproteination (5% NaOH) (Benjakul & Sophandora, 1993). As a result of the incomplete *N*-deacetylation during the production of chitosan, it is referred to as an *N*-deacetylated derivative of chitin (Ravi Kumar, 2000).

The product from depolymerization of chitosan with an average molecular weight of <3900 Da and degree of polymerization (DP) of <20 is known as chitooligosaccharide (Lodhi et al., 2014). The depolymerization of chitosan can be performed by enzymatic degradation, acids, or physical methods (Aam et al., 2010). Chitosanase and other nonspecific enzymes, such as cellulase, proteases, and lipases, can be used for enzymatic depolymerization of chitosan (Lodhi et al., 2014; Qin et al., 2004). Phosphoric, nitrous, hydrofluoric, and hydrochloric acids can be employed for acid hydrolysis (Lodhi et al., 2014; Mourya, Inamdar, & Choudhari, 2011). Irradiation with low-frequency ultrasound (20 kHz) is used as a physical method of hydrolysis. The influence of the method and conditions for depolymerization of chitosan on the molecular weight and degree of polymerization of chitooligosaccharide have been documented elsewhere (Lodhi et al., 2014; Mourya et al., 2011).

Antimicrobial, antioxidant efficacy, and application of chitosan and chitooligosaccharide for seafood preservation

Application of chitosan and chitooligosaccharide as additives, coating agent, or wrapping film is an environmentally friendly approach for the inactivation of microorganisms in seafood, avoiding any adverse impact on the chemical, nutritional, and sensory attributes of these products (Ganguly, 2013). Chitosan acts as an antioxidant, oxygen barrier, or lowers the respiration rate, which can retard the growth of microorganisms in foods (No, Meyers, Prinyawiwatkul, & Xu, 2007). Chitosan and chitooligosaccharide have demonstrated their antimicrobial properties against many bacteria, yeasts, and mold (Laokuldilok et al., 2017; Raafat & Sahl, 2009). Leakage of proteinaceous components and other intracellular constituents caused by interaction between the negatively charged microbial cell membrane and positively charged biopolymer is the major antimicrobial mechanism of chitosan (Kumirska, Weinhold, Thöming, & Stepnowski, 2011; No et al., 2007). Inhibition of mRNA and protein translation, as a result of the interaction between the microbial DNA with the diffused hydrolysis product, and also chelating of essential nutrients, metals, and spore elements are also possible mechanisms (No et al., 2007). The antimicrobial activity of chitosan and chitooligosaccharide is usually stronger against bacteria than mold.

The sensitivity of bacteria to chitosan and chitooligosaccharide is not well elucidated. One study stated that Gram-positive bacteria are less sensitive to the antibacterial effect of these compounds when compared with Gram-negative bacteria (Kong, Chen, Xing, & Park, 2010) but the opposite finding was published by other investigators (Rabea, Badawy, Stevens, Smagghe, and Steurbaut (2003). Likewise, the literature indicates that chitosan has less (Rakkhumkaew & Pengsuk, 2018) and more (Jeon, Park, and Kim (2001) inhibitory action against bacteria and growth of molds than chitooligosaccharide. The disagreement in antimicrobial properties is attributed to the differences in the degree of deacetylation and a molecular weight between chitosan and chitooligosaccharide (No et al., 2007; No, Young Park, Ho Lee, & Meyers, 2002; Tsai et al., 2002). Other influencing factors include the initial microbial load, pH, concentration and type of biopolymer, temperature, and nature of the food (Alishahi & Aïder, 2012; Ganguly, 2013; Kumirska et al., 2011; Lahmer, Jones, Townsend, Baker, & Williams, 2014).

Chitosan and chitooligosaccharide can act as both primary and secondary antioxidants (Agulló, Rodríguez, Ramos, & Albertengo, 2003). The radical scavenging activities of chitosan and chitooligosaccharides against intracellular radicals were reported earlier (Lodhi et al., 2014; Mendis, Kim, Rajapakse, & Kim, 2007). Chitosan and chitooligosaccharides are more effective as a secondary antioxidant, mainly via metal chelation (Agulló et al., 2003). The degree of deacetylation and molecular weight of chitosan and the degree of depolymerization of chitooligosaccharide were the main factors affecting the antioxidant activity (Choe & Min, 2009; Je & Kim, 2012; Nimse & Pal, 2015). To the best of our knowledge, there is no information about the extension of shelf-life of seafood using chitooligosaccharide. However, it has shown promising results in prolonging the shelf-life of bread and meat (Rakkhumkaew & Pengsuk, 2018; Rao, Chander, & Sharma, 2008; Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Due to its film-forming ability, a chitosan solution is widely used as an outer coating to protect seafood from environmental factors and microbial invasion. TBARS, TVB-N content, LAB count, *Pseudomonas* count, and mesophilic bacteria count were lower in brown trout when coated with chitosan prepared in 1.5% acetic acid than 1.5% lactic acid (Alak, 2012). Also, the shelf-life was extended to 9 and 12 days for samples coated with chitosan dissolved in lactic acid and acetic acid, respectively, compared to the control (6 days; Alak, 2012). Günlü and Koyun (2013) studied the impact of an edible film prepared with 2% chitosan dissolved in 1% acetic acid on the quality of seabass fillets stored at 4 °C. TVB-N and TMA-N contents, and psychrotrophic and mesophilic bacteria counts in seabass coated with chitosan-based

edible films were significantly lower than the control during storage, displaying a shelf-life of 27 days versus 3 days for the control. The sensory, microbiological, and chemical qualities of 1 and 2% chitosan-treated sardine fillets were maintained for up to 8 and 10 days, respectively, compared to 5 days for untreated samples (Mohan, Ravishankar, Lalitha, & Srinivasa Gopal, 2012).

Chitosan has been combined with other preservatives or treatments to enhance its preservative effect. Shrimps coated with chitosan dissolved in 1% acetic acid in the presence of 1.5% garlic oil had an extended shelf-life (15 days) when compared to the control (5 days; Aşik & Candoğan, 2014). A synergistic effect of chitosan film coating (1.5% w/v chitosan) with orange peel EO (0.5–2.0%) on the shelf-life of deep-water pink shrimp was demonstrated by Alparslan and Baygar (2017). The shrimp coated with chitosan film incorporated with orange peel EO had a 15-day shelf-life, whereas a shelf-life of 10 days was recorded for shrimp coated with chitosan only. The shortest shelf-life (7 days) was found for the control. Aşik and Candoğan (2014) documented the reduced lipid oxidation and microbial growth in shrimps coated with 3% (w/v) chitosan enriched with garlic oil (up to 1.5%), and it prolonged the shelf-life by 5 days.

Lipid oxidation and microbial counts were lower in oyster treated with ozone and coated with 2% chitosan than the control (untreated sample) (Rong, Qi, Bang-zhong, & Lan-Lan, 2010). The shelf-life was extended to 10–12, 14–15, and 20–21 days for ozonated water-treated samples, chitosan-treated samples, and ozonated water- and chitosan-treated samples, respectively, compared to 8–9 days for the control. Cao, Xue, and Liu (2009) immersed oysters in 5 g/L chitosan solution at a ratio of 1:2 (w/v), which were then wrapped in sterile plastic bags and stored at 5 °C. Chitosan treatment extended the shelf-life of oysters from 8 to 9 days to 14 to 15 days based on microbiological, biochemical, and sensory evaluations. Chitosan coating (2% chitosan dissolved in 1% acetic acid) was applied to whole silver carp stored at −3 °C and its shelf-life was extended to 30 days, compared with 20 days for the control (Fan et al., 2009).

Limitations of chitosan and chitooligosaccharide for seafood preservation

Since chitosan is only dissolved in acidic solution, its adverse effect on the texture and appearance of seafood is inevitable (No et al., 2007). The acidic condition may cause protein precipitation, loss in water-holding capacity, or a sour taste. These alterations lead to rejection by consumers. However, the solubility of chitosan in water is improved by either chemical or enzymatic modification. Chemical modifications, such as graft copolymerization of chitosan with acrylic acid sodium salt, mono (2-methacryloyl oxyethyl)acid phosphate, methylacrylic acid sodium salt, and vinylsulfonic acid sodium salt, have been developed to improve the water solubility of chitosan (Jung, Kim, Choi, Lee, & Kim, 1999; Xie, Xu, Wang, & Liu, 2002). Seafood, like other food materials, is a complex system, consisting of fat, protein, vitamins, salts, carbohydrate, and other constituents. These compounds can interact with chitosan and chitooligosaccharide, leading to the loss of their antioxidant and antimicrobial properties (No et al., 2007).

Bacteriocins

Some Gram-negative and Gram-positive bacteria produce proteins or polypeptides with promising antimicrobial properties. These substances are known as bacteriocins (Zacharof & Lovitt, 2012). Bacteriocins are commonly mistaken for antibiotics. Bacteriocins are primary metabolites synthesized in the ribosome during the primary phase of less growth by bacteria and they exhibit a narrow antimicrobial spectrum, mostly against closely related species. In comparison, antibiotics are secondary metabolites with a wider antimicrobial range (Beasley & Saris, 2004). The arginyl and lysyl residues in bacteriocins make the molecules cationic and amphipathic in nature (Rodrı et al., 2003).

LAB produce a wide number of bacteriocins that can be classified into three classes, based on their biochemical and genetic characteristics: lantibiotics, non-lantibiotics, and bacteriolysins (see Figure 3; Rea, Ross, Cotter, & Hill, 2011; Sahoo, Jena, Patel, & Seshadri, 2016). Bacteriocins are suitable for human consumption because they have a low molecular weight and are easily degraded by the mammalian gastrointestinal tract proteolytic enzymes (Zacharof & Lovitt, 2012). LAB and their primary and secondary metabolites are widely applied in the food industry because they are GRAS (generally recognized as safe) (Rodrı et al., 2003). LAB bacteriocins are reported to possess profound antimicrobial activity against pathogens, such as *L. monocytogenes*, *Campylobacter* sp., and *Clostridium perfringens,* as well as food spoilage microorganisms, such as *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Bali, Panesar, & Bera, 2011; Mehta, Arya, Goyal, Singh, & K Sharma, 2013; Messaoudi et al., 2012; Omar et al., 2013; Svetoch & Stern, 2010).

Production of bacteriocins

Bacteriocins are produced in large quantities with the LAB fermentation process (Bali et al., 2011). Substrates, such as meat extract and yeast extract in commercial media (de Mann–Rogosa–Sharpe), and biopeptone can be used in the production of bacteriocins (Ogunbanwo, Sanni, & Onilude, 2003), although there are limits due to the high cost of these substrates. Bacteriocin production using waste materials such as, whey, marine by-products, molasses, and other industrial wastes, such as potato liquor, grape waste, and fish meal (Bali, Panesar, & Bera, 2016), has gradually created a niche, due to lower cost and also lower impact on environmental pollution (Makkar, Cameotra, & Banat, 2011). Modification of LAB strains and optimization of process parameters, such as temperature, pH, and media composition have been reported to be cost effective and to increase the yield of bacteriocins (Abbasiliasi et al., 2011; Garsa, Kumariya, Sood, Kumar, & Kapila, 2014). The genes responsible for bacteriocin biosynthesis are generally clustered together with structural cognate immunity genes (Perez, Perez, & Elegado, 2015). Bacteriocins are typically synthesized as biologically inactive precursors, containing a modifiable N-terminal leader peptide attached to the C-terminal propeptide (Perez et al., 2015). This inert substance is transformed into an active bacteriocin after undergoing an enzymatic process, known as bacteriocin maturation (Riley & Wertz, 2002), before it is transported out of the cell. The major functions of the leader peptide are to (1) protect the bacteriocin while it is still internalized within the producing strain by ensuring that metabolite is in an inactive state (precursor peptide form), (2) serve as a recognition site that directs the propeptide toward maturation by the biosynthetic enzymes, as well as the transport proteins involved in its translocation outside of the cell, and (3) help ensure that propeptide is in a suitable conformation for interaction with the enzymes during the maturation process (Oman & Van Der Donk, 2010; Perez et al., 2015). Factors affecting bacteriocin production have been reviewed previously (Bali et al., 2016; Chen & Hoover, 2003; Garsa et al., 2014). Siderophores (iron-chelating compound), which is produced by bacteria and fungi used in transporting iron across cell membranes

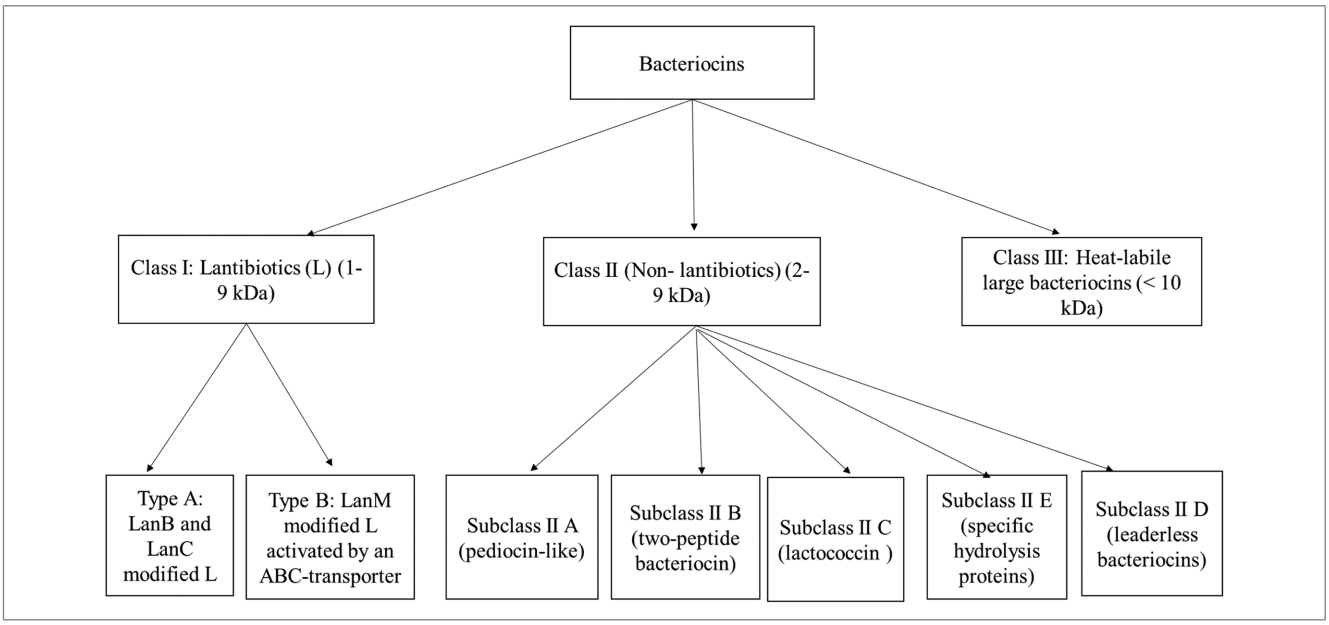

Figure 3–Classification of bacteriocins.

(Gram & Melchiorsen, 1996; Wang, Fu, Bao, & Wang, 2017), is also reported to have a promising antimicrobial activity against Gram-negative bacteria (Ito et al., 2017).

Antimicrobial efficacy and application of bacteriocins for seafood preservation

Bacteriocins display antimicrobial action against various strains of pathogen and spoilage microorganisms via a mechanism specific to each type and class of bacteriocins (Perez et al., 2015). In general, the interaction between the target strain's cell membrane and the (typically cationic) bacteriocins play an important role in its antimicrobial properties. The electrostatic interaction created between the negatively charged bacteria cells and positively charged bacteriocin molecules is necessary for inhibitory effect. However, their ability to kill the bacterial cells relies on the interaction of the bacteriocin with bacterial cell membrane receptor molecules, which vary among the different classes and subclasses of bacteriocins (Pinchas, LaCross, & Dawid, 2015). Class I bacteriocins (lantibiotics) have two antimicrobial mechanisms, namely (1) disruption of the integrity of the bacterial cell membrane by forming pores that lead to impairment of the membrane potential and loss of the intracellular components (Hsu et al., 2004), besides (2) enzyme inhibition, which involves binding to lipid II (the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall), which causes improper cell wall synthesis and thereby cell death (Perez et al., 2015). The mechanisms of antimicrobial properties for class II bacteriocins (non-lantibiotics) differ within the subclasses, but, in general, they cause leakage of cytoplasmic molecules by inducing membrane permeability (Perez et al., 2015; Perez, Zendo, & Sonomoto, 2014). For class I bacteriocins, the receptor or docking molecule is lipid II, while class II bacteriocins interact with the mannose ABC transporter, MptD. However, lacticin Q, a leaderless bacteriocin, does not need a docking molecule (Yoneyama et al., 2009). Instead, high-level membrane permeabilization of target strains without requiring specific receptors is the mechanism for class III antimicrobial action (Yoneyama et al., 2009). For Gram-negative bacteria, this interaction is rare or limited because of their lipopolysaccharide outer membrane. However, the cell integrity of these bacteria was reportedly compromised when bacteriocins were combined with surfactants (Perez et al., 2015).

Bacteriocins have been used to control the growth of pathogenic and spoilage bacteria in fish. The use of bacteriocins along with other preservatives or methods have led to increased efficacy in prolonging the shelf-life of fish and fish products. Bacteriocins produced by *Bacillus* sp. isolated from curd exhibited strong bactericidal potency against *Salmonella* sp. and *Vibrio* sp. in infected marine fish *Parastromateus niger* and squid *Loligo duvauceli* (Ashwitha, Thamizharasan, Vithya, and Karthik (2017). This bacteriocin significantly reduced the total bacterial count of the infected tissues stored at both –4 and –20 °C in a time-dependent manner. Ekhtiarzadeh et al. (2012) verified that the growth of *Vibrio parahaemolyticus* (inoculated at 10^5 CFU/g) and *L. monocytogenes* (inoculated at 10^3 cfu/g) was significantly inhibited in salted fish fillets (4% NaCl) during storage at 4 °C for 21 days, following treatment with nisin (Ni), *Zataria multiflora* Boiss EO, or their combinations. *V. parahaemolyticus* inoculated in the fish was completely inhibited by 0.75 mg/mL Ni + 0.405% EO, 0.045% EO, and 0.75 μg/ml Ni, at days 2, 6, and 9, respectively. In comparison, *L. monocytogenes* increased after treatment with EO and Ni alone but was completely inhibited by combined treatment of EO (0.405%) + Ni (0.25 or 0.75 mg/mL) at 1 day.

At a very low concentration (1183 IU/g), Ni inhibited the growth of Gram-positive *Lactobacillus plantarum* and *Listeria innocua* in fish homogenates stored at 30 °C (Schelegueda, Gliemmo, & Campos, 2012). Higher concentrations (>4300 IU/g) were needed against Gram-negative *P. aeruginosa* and *Pseudomonas fluorescens.* This difference was attributed to the impermeable outer cell membrane of Gram-negative bacteria, which prevents Ni from interacting with the cytoplasmic membrane (Thomas, Clarkson, & Delves-Broughton, 2000). Conversely, a synergistic effect was exerted on *S. putrefaciens* and an additive effect on *L. innocua* by combined treatment of chitosan (300 ppm) and Ni (3000 IU/g).

Ghalfi, Allaoui, Destain, Benkerroum, and Thonart (2006) developed a novel method using cell-absorbed bacteriocin (that is, a suspension of the *Lactobacillus curvatus* CWBI-B28

Table 2–Antimicrobial and antioxidant peptides.

Property	Source	Peptide	Size (MW)	Inhibition activity	Mode of action	Reference
Antimicrobial	Giant squid (*Dosidicus gigas*)	–	≤75 kDa	0.1–1.0 cm inhibition zone against tested Gram-positive and Gram-negative	Membrane disruption and penetration	Mosquera et al. (2016)
	Exocoetus volitans backbone	–	–	1–3 mm inhibition zone against Gram-negative (*Proteus vulgaris*, *Salmonella typhi*, and *Vibrio cholera aerogenes*) and Gram-positive (*Bacillus subtilis*) bacteria	Membrane disruption and penetration	Naqash and Nazeer (2011)
	Porcine liver	–	–	9–16 mm inhibition zone against *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*	Membrane disruption and penetration	Verma, Chatli, Kumar, and Mehta (2017)
	Camel milk casein	–	–	12–18 mm inhibition zone against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*	Membrane disruption and penetration	Kumar, Chatli, Singh, Mehta, and Kumar (2016)
Antioxidant	Shrimp	–	–	ABTS and DPPH radical scavenging activities, ferric reducing antioxidant power	–	Faithong, Benjakul, Phatcharat, and Binsan (2010)
	Croceine croaker (*Pseudosciaena crocea*) muscle	PTSPL, GPGPGL, VGGAP	640.7 Da, 618.89 Da, 484.56 Da	DPPH, superoxide, ABTS, and hydroxyl radical scavenging activities, lipid peroxidation inhibition	–	Cai et al. (2015)
	Rice residue protein	RPNYTDA, TSQLLSDQ, TRTGDPFF, NFHPQ	<3 kDa	DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power	–	Yan, Huang, Sun, Jiang, and Wu (2015)
	Oyster (*Saccostrea cucullata*)	LANAK, PSLVGRPPVGKLTL, VKVLLEHPVL	515.29 Da, 1432.89 Da, 1145.75 Da	DPPH radical scavenging activity, inhibiting human colon carcinoma (HT-29) cell line	-	Umayaparvathi et al. (2014)
	Nile tilapia (*Oreochromis niloticus*)	–	513 Da, 1,484 Da	DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power, metal chelating ability	–	Yarnpakdee et al. (2015)
	Common kilka (*Clupeonella cultriventris caspia*)	–	3–9 kDa	DPPH and ABTS radical scavenging activities	–	Zamani et al. (2017)
	Seabass skin		364 Da	DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power	–	Senphan and Benjakul (2014)
	Grass carp (*Ctenopharyngodon idella*) skin	PYSFK, GFGPEL, GGRP	640.74 Da, 618.89 Da, 484.56 Da,	DPPH, hydroxyl, and ABTS radical scavenging activities, lipid peroxidation inhibition	–	Cai et al. (2015)
	Unicorn leatherjacket (*Aluterus monoceros*)	GPGPVG, LPGPAG, LAGPVG, GGPLG	555.27 Da, 511.29 Da, 557.30 Da, 472.24 Da,	superoxide dismutase and catalase activities	–	Karnjanapratum, O'Callaghan, Benjakul, and O'Brien (2016)
	Seabass (*Lates calcarifer*) skin	GLPGPA, GATGPGGPLGPA), VLGPP, GLGPLGPV	646.36 Da, 1,107.59 Da, 532.31 Da, 780.44 Da	oxygen radical absorbance capacity, ABTS radical scavenging, ferric reducing antioxidant power, ferrous chelating activity		Sae-leaw et al. (2016b)
	Unicorn leatherjacket skin	–	1,170 Da, 750 Da	ABTS and hydrogen peroxide scavenging activities, ferric reducing antioxidant power, ferrous chelating activity	–	Karnjanapratum and Benjakul (2015)

bacteriocin-producing cells on which maximum bacteriocins had been immobilized by pH adjustment) that completely inactivated *L. monocytogenes* in contaminated cold-smoked salmon within 3 days, and no *Listeria* cells were detected up to 22 days. Ye, Neetoo, and Chen (2008) hindered the initial growth of both anaerobic and aerobic microorganisms in cold-smoked salmon stored at 4 °C by wrapping the fish slices with a Ni (500 IU/cm^2)-coated low-density polyethylene film. However, satisfactory inhibition of spoilage organisms during long-term storage required the combined application of Ni (2000 IU/cm^2)-coated treatment onto vacuum-packed smoked salmon. Although bacteriocins are widely applied for the preservation of fish, there is limited information on the bio-preservation of mollusks and shrimp, which could be attributed to the limited development of bacteriocins against seafood pathogens (Bakkal, Robinson, & Riley, 2012; Subasinghe, 2009).

Limitations of bacteriocins for seafood preservation

Low yield and the high cost of production are major constraints restricting the application of bacteriocins in food as bio-preservatives (Makkar et al., 2011). Ogunbanwo et al. (2003) improved the production yield through the addition of meat extract, yeast extract, and peptide to commercial media, including de Mann–Rogosa–Sharpe, yet such media are also costly. As mentioned above, this obstacle can be counteracted by using food-grade media (for example, cheese whey, fish meal, and soybean residue). Purification is a major process in the production of bacteriocins at the industrial or commercial level. A review of the purification protocols highlighted that despite the development of many successful techniques at laboratory scale, more streamlined and cost-effective processes are necessary to generate bacteriocins at an industrial level (Garsa et al., 2014). Bacteriocins singlehandedly extend the shelf-life of seafood based on microbial inhibition. Nevertheless, they cannot lower or prevent lipid oxidation. In this regard, it must be applied along with an antioxidant to extend the shelf-life of seafood more effectively.

Bioactive Peptides

Specific fragments of proteins, also known as bioactive peptides, are not only potential sources of amino acids but also have beneficial physiological functions, such as immunomodulatory (Sae-leaw, O'Callaghan, Benjakul, & O'Brien, 2016a), antihypertensive (Harnedy & FitzGerald, 2012), antithrombotic (Murray & FitzGerald, 2007), antibacterial (Gómez-Guillén et al., 2010), and antioxidant activities (Sae-Leaw et al., 2016b). Moreover, some of these peptides have multifunctional properties (Harnedy & FitzGerald, 2012). Bioactive peptides can be produced from the hydrolysis of proteins using various proteases. Bioactive peptides contain 2–20 amino acid residues, encrypted within the sequence of the parent protein (Chalamaiah, Dinesh kumar, Hemalatha, & Jyothirmayi, 2012). Ngo, Ryu, and Kim (2014) and Sae-leaw et al. (2016b) reported that the bioactivities of a peptide depend largely on the size, conformation, amino acid composition, and sequence.

Production of bioactive peptides

Fish, egg, milk, legumes, cereals, and marine organisms are promising sources or raw materials for the production of bioactive peptides (Samaranayaka & Li-Chan, 2011). Peptides are also produced from underutilized resources and fish processing by-products (Sae-Leaw et al., 2017a). Enzymatic hydrolysis has been widely used to produce bioactive peptides (Zou, He, Li, Tang, & Xia, 2016). The proteinaceous material and specificity of the enzymes are major factors governing the size, amino acid sequence, and bioactivities of bioactive peptides generated (Jo, Khan, Khan, & Iqbal, 2017). Acid and alkaline hydrolysis of protein can also be used to generate peptides. However, these processes are not compatible with food ingredients because they can lead to racemization, loss of tryptophan, and transformation of lysine to lysinoalanine (Harnedy & FitzGerald, 2012).

Enzymatic hydrolysis with the aid of proteases, such as papain, neutrase, flavorzyme, thermolysin, alcalase, pronase, and ficin, is the most reliable and effective method for producing peptides with functional properties as compared with microbial fermentation and hydrolysis by either acid or alkali (Najafian & Babji, 2014). Protein degradation that leads to peptides with increased charge density contributes to increased solubility (Wieczorek, Adamala, Gasperi, Polticelli, & Stano, 2017). Peptides with different biological activities have been recently produced from fish proteins, including seabass (Sae-leaw et al., 2016b; Sae-Leaw et al., 2017b), kilka (Zamani, Madani, Rezaei, & Benjakul, 2017), tilapia (Yarnpakdee, Benjakul, Kristinsson, & Kishimura, 2015), yellow stripe trevally (Klompong, Benjakul, Kantachote, & Shahidi, 2012), and sardine (Bougatef et al., 2010), by enzymatic hydrolysis. Temperature, time, and type and concentration of enzyme are factors affecting the degree of hydrolysis (DH), which is the ratio of the number of the cleaved peptide bonds to the total peptide bonds (Zamani et al., 2017). DH is an important factor in the enzymatic hydrolysis of proteins. Apart from determining the bioactivities of the peptide, DH also affects the yield of peptides produced (Samaranayaka & Li-Chan, 2011).

Antimicrobial, antioxidant mechanism, and application of bioactive peptides for seafood preservation

Several peptides exhibit antimicrobial activity with different modes of action. The antimicrobial function mainly occurs through the interaction of the peptides with the cytoplasmic membrane of the microorganisms (Perez Espitia et al., 2012). Electrostatic interaction between the anionic residue of peptides and charged lipid on the surface of the microorganism inhibit the growth of microbial cells (Malanovic & Lohner, 2016). Hydrophobic residues and flexibility of the peptides in interacting with the microbial membrane are factors determining the efficacy of peptides as an antimicrobial agent (Jenssen, Hamill, & Hancock, 2006). Overall, electrostatic interaction between the peptide and lipopolysaccharides or lipoteichoic acid on the surface of the Gram-negative bacteria and Gram-positive bacteria, respectively, is considered the first step in the antimicrobial mode of a peptide (Perez Espitia et al., 2012). The native cations (Ca^{2+} and Mg^{2+}) from the cell surface are removed by this electrostatic interaction, which facilitates the entry of peptide into the cell (auto-promoted uptake; Powers & Hancock, 2003). The second step is the arrangement of the peptide on the surface of the cytoplasmic membrane, followed by permeation and translocation of the peptide through the cytoplasmic membrane, thereby causing stress and death of cells (Perez Espitia et al., 2012). On the basis of antimicrobial properties, peptides are classified into two major classes: (1) peptides that act on the cytoplasmic membrane, as illustrated above, and (2) those with no action on the cytoplasmic membrane, but exert an antimicrobial activity by movement into the cells, without causing substantial membrane disturbance (Jenssen et al., 2006; Perez Espitia et al., 2012; Powers & Hancock, 2003).

Peptides are also known to possess antioxidant activity, showing excellent metal ion (Fe^{2+}/Cu^{2+}) chelating potential and the ability to inhibit lipid peroxidation, creating a niche for their applications in the food system as a natural antioxidant (Zou

et al., 2016). The prevention of free radical formation or the scavenging ability of active oxygen species and free radicals are the key roles of bioactive peptides as antioxidants in seafood (Irshad, Kanekanian, Peters, & Masud, 2015). Free radicals with high energy, especially hydroxyl radicals, can interact with all 20 amino acids. However, the imidazole-containing amino acid (His), aromatic amino acids (Tyr, Phe, and Trp), and nucleophilic sulfur-containing amino acids (Met and Cys) demonstrated the most reactivity (Elias, Kellerby, & Decker, 2008).

The amino acids in their free state (free amino acid) are not effective as antioxidants, compared to peptides (Samaranayaka & Li-Chan, 2011). Unique physical and chemical properties conferred by amino acid sequences, especially the inability for the radical peptide (after donating a photon for scavenging activities) to initiate further oxidative reactions, are responsible for the higher antioxidant properties in peptides than in free amino acids (Elias et al., 2008). Ranathunga, Rajapakse, and Kim (2006) stated that the molecular weight of a peptide derived from a food source ranges from 500 to 1800 Da, and it contains Leu or Val (hydrophobic amino acid residues) at the N-terminus (Elias et al., 2008). At the water–lipid interphase, the presence of peptides with high hydrophobicity at the lipid phase can facilitate the scavenging of free radicals generated in the system (Ranathunga et al., 2006). Structure–activity relationship, antioxidant properties and assessment, and potential applications of bioactive peptides have been thoroughly documented (Harnedy & FitzGerald, 2012; Samaranayaka & Li-Chan, 2011; Şanlıdere Aloğlu & Öner, 2011; Zou et al., 2016). Both antioxidant and antimicrobial peptides from different proteinaceous sources are presented in Table 2.

Hydrolysates containing peptides can be added as antioxidant or antimicrobial agents directly to seafood. Lipid oxidation (as measured by TBARS, and peroxide value [PV]) and microbial growth were retarded in minced silver carp meat treated with 1.0% and 1.5% fish protein hydrolysate (PH) produced from yellowfin tuna waste using Protamex^{TM} protease, during storage at 4 °C. Also, the shelf-life of the treated minced meat was extended to 12 days relative to 6 days for the control (untreated) (Pezeshk, Ojagh, Rezaei, & Shabanpour, 2017). Li, Luo, You, and Shen (2015) investigated the effect of grass carp protein hydrolysate (PH) on the lipid oxidation of fish mince stored frozen at −10 °C. The addition of 2.0% grass carp PH lowered the increases in PV, TBARS, and formation of conjugated dienes by 51.1%, 34.5%, and 49.7%, respectively, when compared with the control at week 5 of storage.

Limitations of bioactive peptides for seafood preservation

The bitterness of peptides caused by the presence of hydrophobic amino acids (Phe, Gly, Val, Ala, Leu, Trp, Iso, Pro, and Met) at the C- or N-terminal, basic amino acids at the C-terminal, two hydrophobic amino acids at the C-terminal, or low-molecular-weight peptides (<6 kDa) containing one or more hydrophobic amino acids, is one of the obstacles limiting the use of active peptides in seafood (Newman, O'Riordan, Jacquier, & O'Sullivan, 2015). Amino acid composition, DH, number of carbon atoms on the R-group of branched-chain amino acids, location, and concentration determine the bitterness of peptides (Leksrisompong, Gerard, Lopetcharat, & Drake, 2012). Similarly, the application of PHs is limited due to the bitterness, which depends on the enzymes used, as well as the substrates. The bitter-tasting attribute negatively influences the sensory properties of the products (Liu, Jiang, & Peterson, 2013). This limitation is tackled by different methods, such as the use of exopeptidase, the plastein reaction, solvent extraction (ethanol, isopropanol, butanol), macroporous adsorption resin, or a combination of these methods (Leksrisompong et al., 2012).

Conclusion

Several potential antimicrobial agents and antioxidants have been searched and developed for application as natural and safe preservatives for seafood, which is a perishable product with a short shelf-life. These natural preservatives, in either pure or crude form, can serve as alternatives to synthetic additives in preventing chemical, microbiological, and enzymatic deterioration in seafood. Most of the natural preservatives can be obtained from cheap and underutilized resources, enabling the safe and affordable additives to be used in seafood for quality maintenance and shelf-life extension.

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Authors Contributions

O. O. Olatunde collected the data and compiled and wrote the manuscript. S. Benjakul planned, drafted, and corrected the manuscript.

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