Natural Preservatives for Fish

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Fish is extremely perishable as a result of rapid microbial growth naturally present in fish or from contamination. Synthetic preservatives are widely used in fish storage to extend shelf life and maintain quality and safety. Natural preservatives from microorganisms, plants, and animals have been shown potential in replacing the chemical antimicrobials. Bacteriocins and organic acids from bacteria showed good antimicrobial activities against spoilage bacteria. Plant-derived antimicrobials could prolong fish shelf life and decrease lipid oxidation. Animal-derived antimicrobials also have good antimicrobial activities.

Keywords: natural preservatives ; fish ; spoilage mechanisms ; application

1. Introduction

Fish has high protein content and low saturated fat content, which is considered as highly valuable food [1]. In particular, fish is the primary dietary source of omega-3 polyunsaturated fatty acid (PUFA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), both of which are well-known for the anti-inflammatory action and protective effects on cardiovascular disease [2][3][4]. The World Health Organization (WHO) recommends a regular fish consumption of 1–2 servings per week to provide the equivalent of 200–500 mg of omega-3 PUFA [5][6]. According to statistics, fresh and live fish account for about half of the total seafood consumed by human beings. The word “fresh” refers to fish that have not been frozen, including still alive fish as well as kept in the cold but not frozen section or packed in modified atmosphere [7]. Unlike frozen fish, the fresh fish cannot stay as inventory for one month [8]. For consumers, freshness is also often associated with safety, reassurance and superior taste. In most cases, the customers believe that the fish sold in market in China are caught recently. However, it could take more than a week to arrive at these stores. Consumers are still unsure whether the “fresh” product is really fresh or has been frozen and then thawed [9][10].

Fresh fish can easily deteriorate after being captured due to the endogenous enzyme and rapid microbial growth naturally present in fish or from contamination [11]. In the process of fish decay, decomposition of various components and formation of new compounds occur. What is more, changes in composition during fish decay lead to protein degradation and lipid oxidation, as well as changes in fish odor, flavor, and texture. Therefore, it has become inevitable to develop effective treatment methods to extend the shelf life of fish [12]. Soft or mushy texture of fish limits the shelf life, thereby impeding its marketing. During postmortem handling and storage, the holding temperature, oxygen, endogenous or microbial proteases, moisture can result in detrimental changes in the color, odor, texture, and flavor of fish [13][14]. Therefore, fish have traditionally been cooled and stored in flake ice, refrigerated sea water, or ice slurries or they have been preserved by exposure to chemical agents. At the same time, the fishery industry is always looking for new preservation methods to extend the fish shelf life and provide consumers with the best quality in terms of sensory and nutritional levels [15].

In recent years, researchers have put much effort into searching natural preservatives that could inhibit the growth of bacteria and fungi in food. Meanwhile, a growing number of consumers are aware of the potential negative health effects of chemical preservatives, which has prompted the food industry to find natural products used and developed as alternatives. Natural preservatives are available from a variety of sources including plants, animals, bacteria, algae, and fungi [16][17]. Microbial derived preservatives (e.g., bacteriocin), plant derived preservatives (thyme essential oil, tea polyphenols, rosemary extract, etc.), and animal derived preservatives (e.g., chitosan from crab or shrimp shells) have been demonstrated to have antimicrobial or antioxidant properties. In addition, antimicrobial compounds produced by algae and fungal (mushroom) could be served as potential sources of new antimicrobial substances for use as natural preservatives in food.

2. Spoilage Mechanisms

Fish is extremely perishable compared to other muscle foods and will enter into rigor mortis where fish lose their flexibility due to the stiffening of their muscle just a few hours after death [18]. Studies show that the spoilage of fish results from three basic factors: enzymatic autolysis, oxidation, and microbial growth [19].

2.1. Autolytic Enzymatic Spoilage

As the degradation process of fish begins with autolytic enzymatic spoilage, chemical and biological changes take place in dead fish shortly after capture due to autolytic enzymatic breakdown of major molecules. The autolytic enzymes had a significant impact on textural deterioration (softening) and therefore on spoilage; however, they did not produce the characteristic spoilage off-flavors and off-odors [20][21]. Some studies have demonstrated that the fish quality could deteriorate even though comparatively low levels of autolytic degradation were present and limit the shelf life of fish [22][23].

Some gastrointestinal digestion enzymes as well as endogenous muscular enzymes are found in the viscera and muscle of fish after being caught. These enzymes can be conducive to postmortem degradation in fish muscle and fish products during processing and storage, which can lead to a sensory or product associated change. On the other hand, the autolysis of fish muscle proteins could also result in fish meat spoilage and biogenic amines production. The degree of fish freshness has to be determined in the autolysis stage before the spoilage of fish begins [21]. It has been pointed out that low temperature and a_w kept during the storage period can maintain a low activity of the endogenous autolytic enzymes in muscle [24]. In addition, the temperature of vacuum-packed cold-smoked salmon storage should be at low temperature to maintain low native enzyme activity in the tissue [25].

2.2. Oxidative Spoilage

Lipid oxidation could result in spoilage and deterioration and contained a three-stage free radical mechanism: initiation, propagation, and termination [26][27][28]. Some studies suggested that lipid oxidation in fish may be initiated and propagated by a number of mechanisms including auto-oxidation, lipoxygenase, microsomal enzymes, photosensitized oxidation, and peroxidase [29]. In addition, iron-bound proteins in fish muscles, such as hemoglobin, ferritin, myoglobin, hemosiderin, and transferrin, as well as other metals, may be released during storage and play an important role in initiating and/or activating lipid oxidation [30].

Lipid oxidation can be divided into enzymatic and non-enzymatic oxidation, both of which can result in serious decreases in fish qualities. It is typically recognized that oxidation involves the reaction of oxygen with the double bonds of fatty acids. Fish is rich in unsaturated fatty acids and prooxidant molecules, which are extremely susceptible to oxidation resulting in rancidity development and quality loss [31]. These compounds result in deteriorations of smell, color, texture, and nutritional values. Lipid oxidation products also have been demonstrated to promote protein denaturation, modification of protein electrophoretic profiles, nutritional losses, endogenous antioxidant systems losses, and developments of fluorescent compounds [32]. Accordingly, lipid oxidation leads to a decrease of fish acceptability by consumers [33].

2.3. Microbial Spoilage

Fish has high contents of free amino acids, a high post mortem pH, high water contents, and many fish species contain trimethylamine oxide (TMAO), which promote bacterial growth in a wide temperature range [34]. The microbial growth is considered to be the major cause of the deterioration of fish quality, causing up to 25–30% loss of such products [17]. It is generally believed that each fish has its unique flora, which is determined by raw materials, processing parameters, subsequent storage conditions, and microbial tolerance to storage conditions [35]. For example, it was reported that spoilage microorganisms for aerobically stored frozen fish including salmon were species within the genera *Shewanella* (*S.*) and *Pseudomonas* (*P.*), while the CO_2-resistant *Photobacterium (Ph.) phosphoreum* dominated on fish under modified atmosphere packaging [34][35][36].

It is important to distinguish non-spoilage bacteria from spoilage ones as many of the bacteria present do not actually contribute to spoilage during fish storage [37]. A variety of psychrotrophic Gram-negative bacteria comprise the main part of the initial microbiota of fish from temperate sea waters. However, only a small fraction of fish microbiota is responsible for spoilage, known as specific spoilage organisms (SSOs). The SSOs could dominate and produce the metabolites through assimilation of nonprotein-nitrogen of fish muscle with unpleasant and unacceptable off-flavors, which directly affect the organoleptic properties of fish resulting in its rejection by the consumers [38]. The SSOs were different for different fish species and preservation conditions. Research studies demonstrated that *Pseudomonas* spp. was the SSOs for Atlantic salmon (*Salmo salar*) in modified atmosphere packaging and bighead carp (*Aristichthys nobilis*) with 2% salt, whereas *Aeromonas* was the SSOs for unsalted bighead carp [39].

3. Natural Preservatives for Fish

Fresh fish is highly perishable, and even if it can be refrigerated or frozen to extend its shelf life, these processes may not be sufficient to prevent lipid oxidation, rancidity or bacterial growth. In most cases, it is also necessary to improve the quality of fish [40]. For this reason, it is required that preservatives should be properly added to fish during storage.

With the rapid development of social economy, the application of natural preservatives in food has attracted more and more attention from the public. Generally, the public will choose a food with no preservatives, but if these are not available, the same consumer will choose a food containing natural preservatives over synthetic ones [41]. Natural preservatives guarantee that the food is free of microorganisms and safe to eat. Ideally, natural preservatives should have broad bactericidal and fungicidal activities, be non-toxic, be active at low concentrations, impart no flavor or color to food, have no pharmaceutical applications, label friendly, and finally cost effective [42]. Natural preservatives generally come from three sources: microorganisms, animals, and plants. In addition, various bio-active compounds extracted from algae, mushrooms and so on can also provide a potential source of new natural preservatives in the food industry [43].

3.1. Microbial-Derived Compounds

Specific strains of lactic acid bacteria (LAB) produce some inhibitory substances (such as diacetyl, reutericyclin), antifungal compounds (such as phenyl-lactate, propionate, cyclic dipeptides, hydroxyphenyl-lactate, and 3-hydroxy fatty acid), bacteriocins, and bacteriocin-like inhibitory substances, which have antibacterial activity and can be exploited against spoilage bacteria and food-borne pathogens in fish during storage [44]. Some applications of microbial-derived compounds in fish preservation are depicted in Table 1.

Table 1. Survey of literature dealing with bacteriocin employed biopreservation of fish.

Product	Bacteriocin Employed	Reported Effects	References
Skinless blue shark steak	Pediocin ACCEL	*L. monocytogenes*↓ [1]	[45]
Oysters, mussels, clams	BacALP7, bacALP57	*L. monocytogene*s↓, *L. innocua*↓	[46]
Cold-smoked salmon	Divercin V41	*L. monocytogenes*↓	[47][48]
Fresh salmon fillets	Bacteriocin produced by *Lb. pentosus* 39	*A. hydrophila*↓, *L. monocytogenes* ↓	[49]
Reef cod	Enterocin CD1	The total viable count↓	[50]
Reef cod	Bacteriocin PSY2	The total count of spoilage bacteria↓	[51]
Pangasius fish fillets	Bacteriocin 7293	Target microorganisms↓	[52]
Reef cod filets	Bacteriocin GP1	Similar effect with that of sodium benzoate and the nisin B440	[53]
Fish pâté using fresh Nile tilapia	Bacteriocin produced by *L. lactis* 3MT	Vibrio↓	[54]

Product	Bacteriocin Employed	Reported Effects	References
Fresh hake paste	Bacteriocin produced by *E. mundtii* STw38	Native flora of fish paste↓	[55]

[1] Inhibited or decreased.

3.2. Plant-Derived Compounds

EOs are complex mixtures of volatile organic compounds (VOCs) produced as secondary metabolites in plants and frequently responsible for the characteristic odor of plants [56]. They are characterized by two or three major VOCs at fairly high concentrations (20–70%) compared to other VOCs [57]. Some EOs have antimicrobial and antioxidant properties and an increasing demand for natural preservatives has led to EOs as potential alternatives for antimicrobials and antioxidants [58]. EOs have been proved to be effective antimicrobials against some foodborne pathogens including *S. Typhimurium*, *E. coli* O157: H7, *Campylobacter, L*. *monocytogenes*, *S*. *aureus,* and others. Studies show that the efficacy of EOs depends on chemical structure, concentration, matching the antimicrobial activity spectrum with the target microorganism(s), interactions with the food matrix, and application method [59].

It has been observed that some EOs show inhibitory effect on membrane integrity against the tested food-borne pathogenic bacteria [60][61][62][63] (Figure 1). Intracellular material leakage is a general phenomenon results in cell death. The hydrophobic nature of EOs could interfere with bacterial lipid membrane resulting in increased permeability of the cell constituents [64][65], which is in agreement with other phenolic compounds [66][67][68][69]. So far, most studies concerning the antimicrobial action mode of EOs have been carried out on bacteria, while less is known about their effects on molds and yeast. Gram-positive bacteria are generally more susceptible than Gram-negative ones [70]. The cell wall lipopolysaccharides (LPS) of Gram-negative bacteria can create a barrier toward macromolecules and hydrophobic compounds, preventing active compounds in EOs reaching to cytoplasmic membrane [71]. The combinations of EOs with other natural preservatives or even other chemical ones also show positive effects.

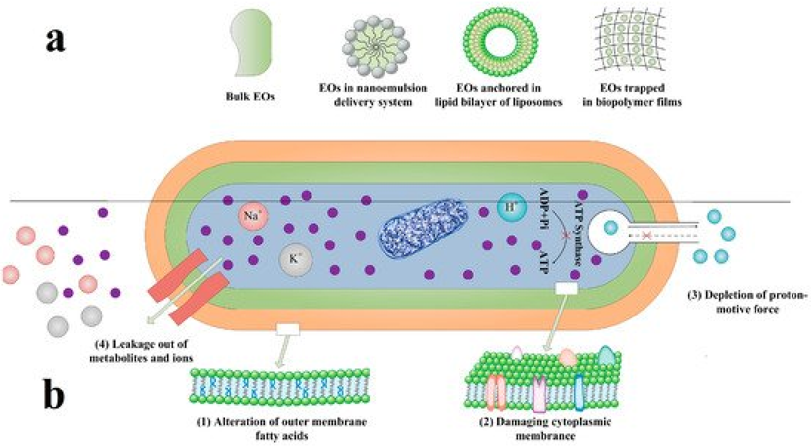

Figure 1. (**a**) Bulk EOs and different types of EO delivery systems, including nanoemulsion, liposomes, and biopolymer films; (**b**) Proposed common mechanisms of action and target sites of essential oils (EOs) or EO delivery systems on bacterial cell [72].

Plant extracts have broad application prospects in fish preservation. The antimicrobial activities of plant extracts may be attributed to the combined effects of polyphenols adsorption to bacterial membrane with membrane disruption and subsequent cellular contents leakage, and the generation of hydroperoxides from polyphenols [73][74][75][76]. Plant extracts also show antifungal activities, antioxidant, antimutagenic activities, and inhibit lipid oxidation in food [77][78][79][80]. Numerous studies have been done in-vitro to evaluate the antimicrobial activities of plant extracts; however, only a few studies are available for fish preservation as the antimicrobial activity of plant extracts does not produce as marked inhibition as many of the chemical preservatives in fish. The plant crude extracts generally contain flavonoids in the form of glycosides, in which the sugar presenting in them decreases the effectiveness against some food-borne pathogens [81][82].

Natural wood smoke is a suspension of vapors, liquid droplets, and solid particles and produced by controlled wood smoldering without oxygen or at reduced oxygen levels. Different woods' smoke have different antimicrobial properties as the woods generate different levels of antimicrobials, such as organic acids, phenols, and carbonyls during pyrolysis.

Now, more than 20 different kinds natural wood smoke including redwood, black walnut, hickory, birch, white oak, aspen, chestnut, and cherry have been evaluated the antimicrobial properties against *A. hydrophila,* and *S. aureus* [83]. Additionally, the smoke treatment could increase redness of the fish muscle and stabilized it during frozen storage [84]. It should be noted that wood smoke contains some harmful compounds, such as polycyclic aromatic hydrocarbons (PAHs) [85][86]. Since the 1970s, liquid smoke has been developed and become popular resulting from the concern of potentially carcinogenic benzopyrenes [87]. Liquid smoke preparations can be either incorporated as a surface additive during post thermal processing or a formula ingredient during batter mixing to reduce or eliminate food-borne pathogens as well as impart desired smoky flavor to the products. Many studies have been focused on the use of liquid smoke as a postlethality dip or spray treatment to reduce or eliminate food-borne pathogens on fish products. Antimicrobial efficacy of liquid smoke can be enhanced by vacuum-packaging, essential oils, and NaCl [88][89][90][91][92]. Also, wood smoke can be converted into nanocapsules using chitosan and surface contact area could be increased, which delayed microbial growth in fish fillets at cold storage conditions [93][94].

As natural sources of bioactive compounds, algae and mushrooms have a wide range of biological activities including antimicrobial, antioxidant, antiviral, anti-inflammatory, and other health promoting benefits [56][95][96][97][98][99]. Among the major bioactive ingredients of algae and mushrooms with demonstrated antimicrobial activities, proteins, antioxidants (polyphenols, flavonoids, and carotenoids), polyunsaturated fatty acids, and polysaccharides are the most important ones [100]. Until now, the antimicrobial potential of algae and mushrooms has been generally tested in vitro, providing reliable quantitative estimates of minimum inhibitory concentration (MIC) values for many samples [100][101]. Compounds reported to be present in algae included phlorotannins, terpenoids, phenolic compounds, acrylic acid, steroids, cyclic polysulphides, halogenated ketones and alkanes, and fatty acids that act as bactericidal agents [102]. The presence of these compounds suggests alternative mechanisms for antimicrobial action. For example, phlorotannins could inhibit the oxidative phosphorylation and bind with bacterial proteins including enzymes and cell membranes, leading to cell lysis [103]. The mechanisms of sulphated polysaccharides and algal polysaccharides may be related to glycoprotein receptors on the cell surface of polysaccharides which bind with compounds in the cell wall, cytoplasmic membrane, and DNA, increasing the cytoplasmic membrane permeability, protein leakage, and binding of bacterial DNA [104]. The antimicrobial activities of mushrooms may be related to a variety of secondary metabolites with biological activity, such as gallic acids, some phenols, volatile compounds, free fatty acids, and their derivatives [105]. Considering the wide biodiversity of mushrooms, they could easily become accessible sources of natural preservatives. However, few studies have evaluated the antimicrobial activities of algae and mushrooms in fish preservation.

Saponins are natural glycosides compounds in some plants showing promising results as a broad-spectrum antimicrobial and antifungal activities [106][107]. The antifungal activity of saponins interacts with cytoplasmic membrane sterols, the ergosterol, can provoke pores and loss of membrane integrity, resulting in cell death [108].

Flavonoids are ubiquitous in photosynthesizing cells and are commonly found in some plant parts. They exhibit broad-spectrum antimicrobial activities due to the ability to form complexes with extracellular and soluble proteins as well as with bacterial membranes [109][110][111]. Flavonoids have antimicrobial activities against bacteria and the hydroxyls at special sites on the aromatic rings of flavonoids improve the activity. However, the methylation of the active hydroxyl groups generally decreases the activity. The hydrophobic substituents such as prenyl groups, alkylamino chains, alkyl chains, and nitrogen or oxygen containing heterocyclic moieties usually enhance the activity for all the flavonoids [112]. As a whole, it is necessary to further investigate their potential use as food preservatives as people are increasingly interested in finding more natural alternatives.

3.3. Animal-Derived Compounds

At present, many animal-derived antimicrobial compounds have also been used for fish preservation. Such examples include chitosan from shellfish, lactoperoxidase, and lactoferrin from milk, and lysozymes from hen eggs [113]. However, one of the major problems associated with animal-derived antimicrobials is their allergen risk; the sources of such ingredients are often allergen-containing foods including shellfish, milk, and egg [114].

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