

Antimicrobial seafood packaging: a review

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Abstract Microorganisms are the major cause of spoilage in most seafood products; however, only few microbes, called the specific spoilage organisms (SSOs), contribute to the offensive off-flavors associated with seafood spoilage. In food, microbial degradation manifests itself as spoilage, or changes in the sensory properties of a food product, rendering it unsuitable for human consumption. The use of antimicrobial substances can control the general microflora as well as specific microorganisms related to spoilage to provide products with higher safety and better quality. Many antimicrobial compounds have been evaluated in film structures for use in seafood, especially organic acids and their salts, enzymes, bacteriocins; some studies have considered inorganic compounds such as AgSiO₂, zinc oxide, silver zeolite, and titanium oxide. The characteristics of some organic antimicrobial packaging systems for seafood and their antimicrobial efficiency in film structures are reviewed in this article.

Keywords Seafood · Microbial hazards · Antimicrobial · Packaging

Introduction

Seafood can undergo rapid microbial contamination and growth if subjected to inadequate handling and storage. One-fourth of the world's food supply and 30 % of landed

fish (Amos 2007) are lost through microbial activity alone. Around 4–5 million tons of fish are lost every year because of enzymatic and microbial spoilage due to improper onsite storage. The specific spoilage microorganisms known to be involved in seafood spoilage are listed in Table 1, in descending order of spoilage activity (Ghaly et al. 2010). Some microorganisms cause spoilage to different degrees depending on the total microbial flora, fish quality, handling and packaging methods, and storage temperature. The traditional methods of fish preservation include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging, and addition of antimicrobial agents or salts. Unfortunately, some of these techniques cannot be applied to some fish products such as fresh meat and ready-to-eat products.

An antimicrobial agent is a chemical preservative that can be incorporated into a packaging material to induce antimicrobial activity. Various antimicrobial agents, which can generally be divided into three major groups, namely, chemical agents, natural spices, and probiotics, can be incorporated into conventional food packaging systems and materials to create new antimicrobial packaging systems (Han 2005). Antimicrobial packaging is a form of active packaging that interacts with the product or headspace between the package and food to obtain a desired outcome (Brody et al. 2001).

In particular, antimicrobial packaging is a promising form of active food packaging for fish products. Microbial contamination of these foods occurs primarily on the surface because of post-processing handling, attempts have been made to improve safety and to delay spoilage by using antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods has limited benefits because the active substances are often neutralized on contact or diffuse rapidly from the surface into the food mass (Quintavalla and Vicini 2002). On the other hand, incorporation of bactericidal

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Table 1 Spoilage-causing microorganisms in seafood

Product	Spoilage activity	Specific spoilage bacteria	Spoilage compounds
Fresh chilled products stored in air	High	<i>Shewanella putrefaciens</i> , <i>Pseudomonas (Alteromonas) putrefaciens</i> , <i>Pseudomonas (altreomonas), fluorescens</i> <i>Fluorescent pseudomonads</i>	TMA, H ₂ S, CH ₃ SH, (CH ₃) ₂ S, HX
Fresh fish, >10 °C	High	<i>Vibrionaceae</i> , <i>Aeromonas</i>	Ketones, aldehydes, esters, non -H ₂ S sulphides
Fresh fish, chilled, MA packed	Moderate	<i>Photobacterium phosphoreum</i> , <i>Moraxella</i> , <i>Acinetobacter</i> and <i>Alcaligene</i>	TMA, HX
Chill stored vacuum-packaged cold-smoked	Low	<i>Lactobacillus</i> , <i>Aerobacter</i> , <i>Flavobacterium</i> , <i>Micrococcus</i> , <i>Bacillus</i> and <i>Staphylococcus Mesophilic</i>	NH ₃ , acetic, butyric and propionic acid

Source: Adopted and modified from Ghaly et al. 2010

TMA Trimethylamine, H₂S Hydrogen sulfide, CH₃SH Methyl mercaptan, (CH₃)₂S Dimethylsulfide, HX Hypoxanthine, NH₃ Ammonia

or bacteriostatic agents into fish formulations may result in partial inactivation of the active substances by the product constituents and therefore, expected to exert only limited effects on the surface microflora. During the first stage of spoilage, fish emit a very bad odor that is undesirable to most consumers; therefore, antimicrobial agents that can reduce this odor are useful to control this problem while enhancing the shelf life of the fish at the same time (Ghaly et al. 2010). Traditionally, the antimicrobial agents are added directly to foods; however, many substances in the food itself inhibit antimicrobial activity, diminishing their effectiveness. In such cases, the use of antimicrobial films or coatings can be more efficient because they can selectively and gradually migrate from the package onto the food surface (Ouattara et al. 2001). Packaging films containing antimicrobial agents pose a potential solution to reduce the challenges of spoilage. The purpose of this paper is to provide an overview of the published data on the antibacterial activity of organic and inorganic components that can be considered suitable for application in seafood and to describe their possible modes of action.

Major microbial hazards

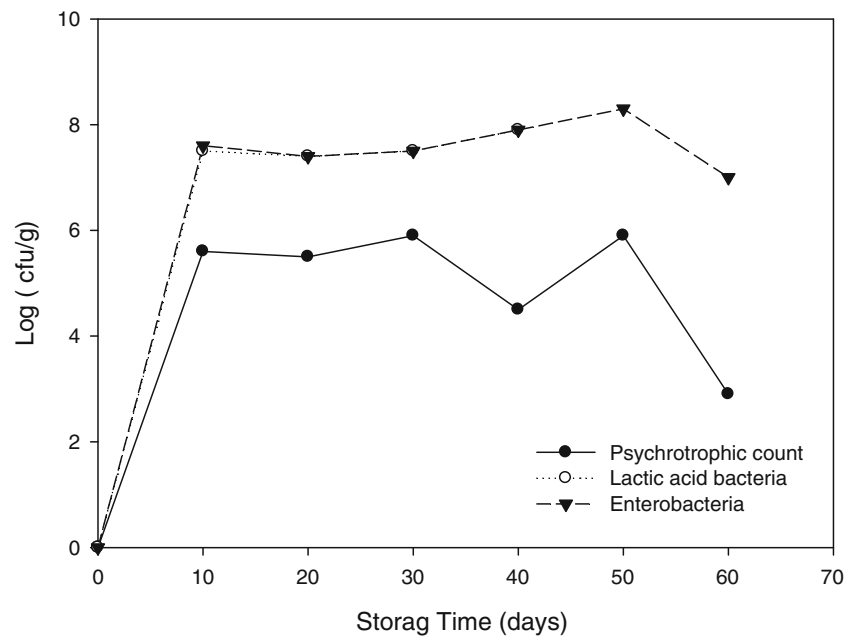
Seafood is associated with many microbial hazards. Temperature shifts during transportation, from low to high or high to low, can cause the formation or reproduction of heat-stable toxins. *Salmonella* species is the main cause of seafood-borne bacterial illness as well as the detention of imports at the border (Norhana et al. 2010). The growth factors associated with *Salmonella* species include the following: temperature range of 5.2–47 °C, pH range of 3.7–9.5; many *Salmonella* strains can survive freezing for up to 9 months (Kerry 2012). *Listeria monocytogenes* is not as widespread as *Salmonella* species but is commonly observed in

seafood because of its presence as an environmental inhabitant in processing facilities. *L. monocytogenes* also grows in the temperature range of –0.4 to 45 °C; it grows rapidly and reaches higher numbers in shrimp and catfish (Shineman and Harrison 1994). *Vibrio* species is also associated with seafood-borne diseases and the most virulent species are *Vibrio vulnificus*. Schwarz (2000) found that the oysters harvested off the Texas coast subjected to rapid cooling showed 97.8 % reduction in the number of *V. vulnificus*, whereas conventionally cooled oysters took four days to reach the same numbers. The toxin produced by *Clostridium botulinum*, a spore-forming anaerobe, is a big concern because it is heat stable, highly toxic, and can grow at temperature exceeding 3.3 °C; the level of *C. botulinum* in seafood has been estimated at 1 to 2400 spores per kilogram of seafood (Dodds 1992). *Aeromonas hydrophila* is more commonly associated with or is exclusively found in seafood and in fresh-flowing, stagnant, and brackish water (Bremer et al. 2003). A study by Papadopoulou et al. (2007) showed that local seafood harvested within 24 h contained *A. hydrophila* as the predominant organism (freshwater fish, 38 %; shellfish, 73–86 %; and marine finfish, 93 %).

The packaging of seafood products has historically been passive, or used to protect from oxygen, desiccation, and microbial contamination. Tamper-evident packaging became the norm 30 years ago. Vacuum-packed cold-smoked salmon containing high levels (10⁷–10⁸ CFU/g) of lactic acid bacteria remain good for several weeks before the product is rejected by sensory testing, which shows that the often used “total bacterial count” does not serve any purpose as a spoilage indicator for this type of product (Fig. 1) (Hansen et al. 1995).

Antimicrobial packaging materials have potential as an alternative solution to prevent the growth of spoilage and pathogenic microorganisms.

Fig. 1 Changes in total psychrotrophic count, lactic acid bacteria and *Enterobacteriaceae* during storage of vacuum-packed cold-smoked salmon (4.6 % w/w in water phase) at 5 °C. Source: Adapted and modified from Hansen et al. 1995



Antimicrobial agents

Antimicrobial packaging have attracted the seafood industry because its potential in the hurdle technique used for minimally processed food. For application in food, seafood, pharmaceuticals, and cosmetic products, the antimicrobial agent industry must follow the guidelines and regulations of the country in which they are used, such as the FDA and/or the EPA in the United States. Thus, new antimicrobial packaging materials can be developed only by using agents that are approved by authorizing agencies such as the FDA as compounds notified-to-be-used within the concentration limits for enhancement or preservation of food safety. Various antimicrobial agents may be incorporated in the packaging system, including chemical antimicrobials, antioxidants, biotechnological products, antimicrobial polymers, and natural antimicrobials. Both organic and inorganic chemical antimicrobial agents are most commonly used in the industry.

Organic antimicrobials

Organic acids such as benzoic acids, parabens, sorbates, sorbic acid, propionic acid, acetic acid, lactic acid, medium-sized fatty acids, and/or their mixture possess strong antimicrobial activity and have been used as food preservatives, food contact substances, and food contact material sanitizers Han (2003). Coma et al. (2001) studied the moisture barrier and the antimicrobial properties of HPMC-fatty acid films (30–50- μ m-thick) containing nisin (105 IU/mL) as the AM agent and its efficacy against *Listeria innocua* and *Staphylococcus aureus* in food products.

The mechanism(s) by which organic acids inhibit microorganisms has been studied extensively. There is little evidence that organic acids influence cell wall synthesis in prokaryotes or that they significantly interfere with protein synthesis or genetic mechanisms. Instead, organic acids are more likely act at the cytoplasmic membrane level. The un-dissociated form of organic acids penetrates the cell membrane lipid bilayer, and once inside the cell, it dissociates because of the higher pH inside the cell. Because bacterial cells should be able to maintain the internal pH near neutral, the protons generated from the dissociation of organic acids must be transported to the exterior. Since the protons generated by the organic acids inside the cell must be extruded using energy in the form of ATP, a constant influx of these protons will eventually deplete cellular energy (Davidson et al. 2002).

The natural antimicrobials and essential oils found in seafood are very helpful against microbial growth and do not have any negative impact on the environment. Consumers demand premium quality seafood without any synthetic additives and there is greater demand by retail outlets for extended shelf life in case of fresh and processed seafood. These reasons have prompted the use of organic antimicrobial agents and there are many ongoing studies relating these agents and specific spoilage microorganisms (Irkin and Esmer 2015) (Table 2).

According to López-Malo et al. (2000), and Nychas and Tassou (2002), the active antimicrobial substances in many spices and essential oils interact with food components, and if used as the only preservative in foods, more than 1 % (w/w) of these spices and essential oils can be required to extend the shelf life of seafood. These high levels often extend a very strong flavor and are primarily useful in sauces and products

Table 2 Activity of organic and inorganic antimicrobial agents against seafood contaminants

Microorganism	Organic antimicrobial agent	Concentration	Reduction (%) in bacterial count	References
<i>Shewanella putrefaciens</i>	Oregano EOs	1–2 g		Tassou et al. 1996
	Cinnamaldehyde	1–4 %	Growth inhibition	López et al. 2007
	Olive oil, lemon juice	1–2 g	Completely inhibition with MAP	Tassou et al. 1996
	Carvacrol	1–4 %		López et al. 2007
<i>Pseudomonas spp.</i>	Thyme	0.1	>44	Kykkidou et al. 2009
	Oregano EOs	1–4 % w/w		López et al. 2007
	Thymol	1000 ppm	Significant inhibition	Mastromatteo et al. 2010
<i>Listeria monocytogenes</i>	Potassium sorbate	2–3 % w/v		Limjaroen et al. 2003
	Oregano EOs	800 ppm	Significant inhibition	Seaberg et al. 2003
		0.8 % v/w	Growth reduction, by 2–3 log ₁₀ units	Tsigarida et al. 2000
	Cinnamaldehyde	1–4 %	Growth inhibition	López et al. 2007
	Chitosan	4.5 mg/cm ² 0.6 mg/cm ²		Ye et al. 2008
Psychrotrophic and anaerobic bacteria	Sodium acetate	2 %		Zhuang et al. 1996
	Nisin	20 g/L	Suppression of coliform and bacterial growth	Kim et al. 2002
	Lacticin NK24	20 g/L	Growth inhibition	Kim et al. 2002
<i>Clostridium sporogenes</i>	<i>Origanum vulgare</i>		>90.0	Dorman and Deans 2000
	<i>Pelargonium graveolens</i>		7.8 ± 0.6 (ZOI) ^a	Dorman and Deans 2000
	<i>Piper nigrum</i>	15 µL	8.7 ± 0.3 (ZOI)	Dorman and Deans 2000
	<i>Syzygium aromaticum</i>		13.4 ± 0.5 (ZOI)	Dorman and Deans 2000
	<i>Thymus vulgaris</i>		>90.0	Dorman and Deans 2000
	Basil	0.100	>85	Mejlholm and Dalgaard 2002
	Bay	0.100	>85	Mejlholm and Dalgaard 2002
<i>Photobacterium phosphoreum</i>	Clove	0.050	>57	Mejlholm and Dalgaard 2002
	Lemongrass	0.005	>85	Mejlholm and Dalgaard 2002
	Marjoram	0.100	>85	Mejlholm and Dalgaard 2002
	Oregano	0.010	>85	Mejlholm and Dalgaard 2002
	Sage	0.075	>85	Mejlholm and Dalgaard 2002
	Thyme	0.050	>85	Mejlholm and Dalgaard 2002

^a (ZOI) - Zone of inhibition

that are mixed with other food ingredients. Mejlholm and Dalgaard (2002) found that 0.05 % (v/w) oregano oil yielded a distinctive but pleasant flavor to cod fillets, and the oil significantly extended the shelf life and delayed spoilage reactions. Oregano oil and many other essential oils are relatively cheap, and the addition of 0.05 % essential oil (v/w) constitutes approximately 1 % of the raw material cost in case of cod

fillets. Majority of the studies focused on the use of essential oils to develop edible coating, encapsulation, or any other form, as we know that high fat content of the fish affects the function of the essential oil. For example, 0.5 µL/g oregano oil is more effective against *P. phosphoreum* on cod fillets than salmon, which is a fatty fish (Mejlholm and Dalgaard 2002). Alboofetileh et al. (2016) reported that alginate-clay films

enriched with 1 % Marjoram essential oil significantly delayed the growth of *L. monocytogenes* during the 15-day storage with final counts reaching 6.23 log CFU/g. Alginate/carboxyl methylcellulose coating+clove essential oil 1.5 % showed lowest ($p < 0.05$) and acceptable biochemical, bacteriological and sensory characteristics attributes up to 16 days storage at 4 °C (Jalali et al. 2016).

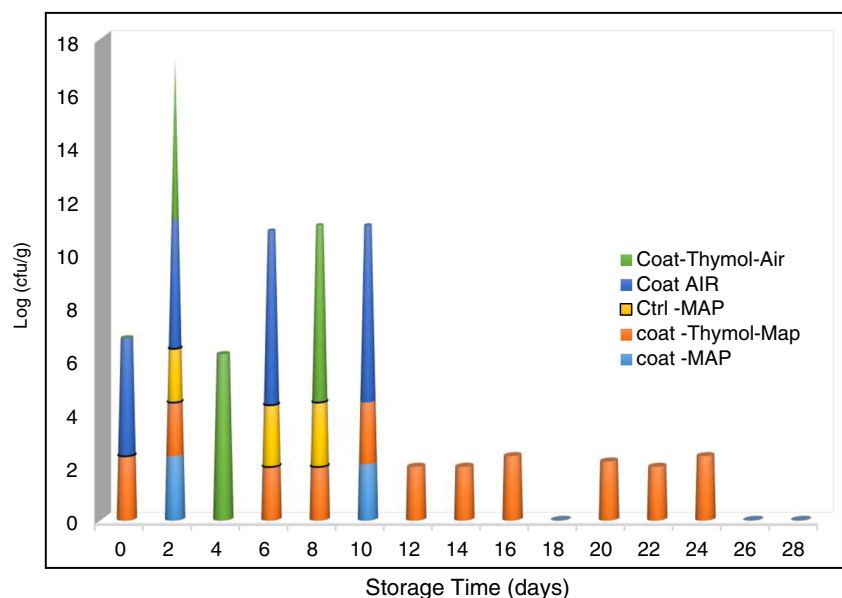
Thymol, one of the key components of oregano oil, is mainly responsible for its antimicrobial activity. Mastromatteo et al. (2010) reported that a single coating did not affect microbial growth on ready-to-use peeled shrimps. A slight antimicrobial effect was obtained when the coating was loaded with thymol. Moreover, the active coating was effective at diminishing the loss in the sensory quality of the investigated product, which was particularly true at the lowest thymol concentration (Fig. 2). It is worth noting that for all MAP samples, the *Pseudomonas* count was always below the detection level until day 26 (Coat-thymol-MAP).

Thymol and carvacrol are the most active constituents of thyme essential oil with a wide spectrum of antimicrobial and antioxidant properties (Burt 2004 and Lambert et al. 2001). Kykkidou et al. (2009) concluded that thyme oil and MAP were the most effective methods to inhibit pseudomonas and H_2S -producing bacteria in swordfish. The shelf life of fresh refrigerated Mediterranean swordfish was 8 and 13 days under aerobic and MAP conditions, respectively. The addition of 0.1 % thyme essential oil extended the shelf life under aerobic conditions by 5 days, whereas the combination of MAP and thyme oil resulted in significant shelf life extension of the swordfish fillets by approximately 7 days. Galotto and Ulloa (2010) showed that flexible plastic films containing thymol as the antimicrobial agent for salmon packaging extended shelf life 18 days at 2 °C. Erkan (2012) concluded that hot smoked

trout treated with thyme and garlic oil maintained a shelf life of 7 weeks in cold storage compared to 5 weeks for untreated hot smoked rainbow trout, based on sensory, chemical, and microbiological evaluation. Karakaya et al. (2015) carried out experiments on whey protein isolate coating enriched with thyme essential oil (3 %, 5 %, and 7 %, v/v) on whole trout under refrigerated storage at 4 ± 2 °C and concluded that increasing amount of thyme oil increased the storage stability of trout, in all aspects. Guran et al. (2014) investigated the effects of different concentrations of thyme, clove, and rosemary extracts on the microbiological, chemical, and sensory attributes of bonito fish patties and found that the addition of essential oils exerted a positive effect on the shelf life of this product; in particular, rosemary essential oil produced a remarkable effect. The mechanism of action of thymol is very similar to that of carvacrol, with both having the hydroxyl group at a different location on the phenolic ring. Both substances appear to make the cell membrane permeable (Lambert et al. 2001). Carvacrol and thymol are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP. The presence of magnesium chloride has been shown to have no influence on this action, suggesting a mechanism other than chelation of cations in the outer membrane.

Chitosan is a nontoxic, biodegradable, and biocompatible natural polymer. It is a good choice for antimicrobial films because of its superior film-forming properties, ability to adsorb nutrients used by bacteria, and capacity to bind water and inhibit various bacterial enzyme systems (Darmadji and Izumimoto 1994). Chitosan, which is mainly obtained from crustacean shells, is the second most abundant natural polymer in nature after cellulose. Therefore, chitosan, which is

Fig. 2 Evolution of mesophiles and hydrogen sulphide-producing bacteria (Log cfu/g) for Step II peeled shrimps. Source: Adapted and modified from Mastromatteo et al. 2010



commercially produced mostly from marine sources (e.g., crustacean shells), has been used to stabilize seafood-based products. Chitosan-based films and coatings have been used for a variety of fish species to reduce microbial flora and to improve overall fish quality and prolong storage life (Duan et al. 2010b). Cao et al. (2009) reported that 5 g/L chitosan extended the shelf life of oysters (*Crassostrea gigas*) from 8–9 days to 14–15 days. Preservative treatment was undertaken by immersing the oysters in 5.0 g/L chitosan solution for 10 min at a ratio of 1:2 (w/v). They explained that *Pseudomonas* and *Shewanella* species are the most prolific microorganisms during the cold storage of fish and shellfish. Günlü and Koyun (2013) found that the shelf life of chitosan film-wrapped and vacuum-packaged sea bass samples ended at 25–30 days, while that of vacuum-packaged sea bass sample alone ended within 5 days. Fernández-Saiz et al. (2013) demonstrated that the incorporation of chitosan acetate film before packaging led to a decrease in the final bacterial population by 1.6 and 3.8 log units in chitosan air-packaged and chitosan vacuum-packaged samples, respectively. Jasour et al. (2015) determined that trout fillets treated with chitosan and lactoperoxidase had significantly lower numbers of *Shewanella putrefaciens*, *Pseudomonas fluorescens*, and psychrotrophic and mesophilic bacteria and found that their shelf life was extended by at least 4 days, compared to the control samples. Speranza et al. (2013) built a polynomial model and highlighted that *P. fluorescens* was the most resistant microorganism in seafood. They also showed that *P. fluorescens* could be inhibited in fillets through an active solution containing 2 % chitosan and 6000 ppm thymol and grapefruit seed extract, combined with packaging under 5:95 O₂/CO₂, maintained fillets at the maximum level of microbiological quality for at least 8–10 days and the sensory attributes were at acceptable levels for about 20 days. The exact mechanism of the antimicrobial action of chitin, chitosan, and their derivatives is still unknown, but different mechanisms have been proposed. Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents. Chitosan acted mainly on the outer surface of the bacteria. At a lower concentration (<0.2 mg/mL), the polycationic chitosan does probably bind to the negatively charged bacterial surface to cause agglutination, whereas at higher concentrations, the larger number of positive charges may have imparted a net positive charge to the bacterial surface to keep them in suspension. Chitosan interacts with the cell membrane to alter cell permeability (Rabea et al. 2003).

Ahmad et al. (2012) concluded in their study that films incorporated with lemongrass oil exhibited more antibacterial activity than bergamot-treated films against *Escherichia coli*, *L. monocytogenes*, *Pseudomonas aeruginosa*, *S. aureus*, and *S. typhimurium*. Shakila et al. (2015) showed that vacuum-

packed fish steaks coated with chitosan and clove films extended the shelf life from 4 to 8 days at 4 °C. Norhana et al. (2012) determined that fresh shrimp in 3 % potassium sorbate (PS), sodium benzoate (SB), sodium diacetate (SD), and combined nisin–EDTA–PS, –SB or –SD solutions resulted in marginal reduction in the number of inoculated *L. monocytogenes* at day 0 and 3 at 4 °C. However, the same treatments failed to reduce the number of *Salmonella* spp. on the shrimp surface. Neetoo and Mahomoodally (2014) found that cellulose-based coatings incorporating nisin, potassium sorbate, or sodium benzoate reduced the population of *L. monocytogenes* in cold-smoked salmon by a maximum of 4.2, 4.8, and 4.9 log CFU/cm², respectively, after 4 weeks of refrigerated storage. Behnam et al. (2015) found that treatment of the vacuum packaged rainbow trout with nisin resulted in improvement of quality and extension of shelf life of the fish from 12 to 16 days at 4 °C. The cytoplasmic membrane of vegetative cells is the primary site of action of nisin. Nisin is believed to induce pore formation in the cytoplasmic membrane, which results in the depletion of proton-motive force and loss of cellular ions, amino acids, and ATP.

Ramírez-Suárez et al. (2015) determined that vacuum-packed frankfurters made from jumbo squid (*Dosidicus gigas*) muscle with the antimicrobial agent Pronat (0.1 %) were physico-chemically stable and in good microbiological condition up to 21 days. Pang et al. (2013) demonstrated that allyl isothiocyanate (18 and 36 µg/L) present in the vapor phase was an effective antimicrobial agent to inhibit the growth of *P. aeruginosa* because it provided a 3-fold longer shelf life of fresh catfish fillets relative to the controls. Higher gaseous AIT concentrations showed an increased antimicrobial effect on *P. aeruginosa*. Karakaya et al. (2015) used potassium sorbate and sodium lactate either separately or in combination with 3 % (w/v) brine for pre-smoking or by spraying post-smoking on rainbow trout (*Oncorhynchus mykiss*) fillets and determined that potassium sorbate in brine used at the pre-smoking stage was most efficient, which could maintain trout fillets within the range of consumable limits, for 4 weeks at 6 ± 1 °C. Pre-smoking reduced the total aerobic mesophilic bacteria (TAMB). Ozogul et al. (2014) determined that vacuum-packed European eel had a 16- and 20-day shelf life with laurel and myrtle treatment, respectively. Natural extracts from myrtle and laurel can be used in the food industry to extend the shelf life of seafood because they exhibit promising antioxidant and antimicrobial effects. García-Soto et al. (2015) found that megrim (*Lepidorhombus whiffiagonis*) wrapped with polylactic acid film containing 8 % alga and 1 % sorbic acid was acceptable after day 11, while control fish specimens that were maintained under polyethylene film alone were rejected at that time. The mechanism by which sorbic acid inhibits microbial growth may partially be attributed to its effect on enzymes. Melnick et al. (1954) postulated that sorbic acid inhibited the dehydrogenases involved in fatty

acid oxidation. Addition of sorbic acid resulted in the accumulation of β -unsaturated fatty acids that are intermediate products of fatty acid oxidation by fungi. This prevented the function of dehydrogenases and inhibited metabolism and growth.

Lee et al. (2015) developed a mathematical model to predict the antimicrobial film-coating requirements for obtaining protection against *L. monocytogenes* in smoked salmon by using a defatted mustard meal-based antimicrobial edible film (DMM film). They found that the film with 0.41 mg/g thiocyanate was predicted to provide 19.7 h of protection in smoked salmon against continuous-post-contamination by *L. monocytogenes* at 4.0 log CFU/g, during which time thiocyanate remained above the inhibitory concentration of 0.28 mg/g. For 24-h inhibition, the initial concentration of thiocyanate required was estimated as 0.43 mg/g in a 0.14-mm-thick film for optimal antimicrobial effects. Fernández-Pan et al. (2015) studied the kinetics of carvacrol release rate toward different food-simulating solvents, and recommend the use of edible films obtained from chitosan with a low molecular weight to preserve medium and high fat content fishes such as sardines, salmon, and tuna. The films showed antimicrobial effectiveness against the gram-negative bacteria *Pseudomonas fragi*, *S. putrefaciens*, and *A. hydrophila*, common spoilers of fish and seafood products. El-Sayed et al. (2015) controlled the growth of *Bacillus cereus/thuringiensis* and *Citrobacter freundii* in fresh chilled Atlantic salmon (*Salmo salar*) by using rosemary plant extracts (*Rosmarinus officinalis*) and showed strong antibacterial activity against *C. freundii*, FIM-SH, and *B. cereus/thuringiensis*, with zone of inhibitions with a mean diameter of 16 and 29 mm, respectively. Patel (2015) also showed that essential oils from many floras could be recommended as safe biopreservatives for fish. Hasani et al. (2015) showed that silver carp (*Hypophthalmichthys molitrix*) fillets treated with grape pomace extract (0 %, 2 %, and 4 %) during chilled storage has lower TVB-N (24.2 and 21.2 mg N/100 g, respectively), TVC (7.33 and 7.09 log CFU/g, respectively), and PTC (7.26 and 7.03 log CFU/g, respectively) at the end of the storage period.

Inorganic antimicrobials

Antimicrobial packaging with inorganic nanoparticles has recently been gaining popularity because of the excellent ability of these materials to withstand harsh process conditions such as high pressure or temperature during the plastic fabrication process, which is of particular importance for seafood due to the difficulties encountered during processing, packaging, and transport. Organic antimicrobial agents are limited in their activity during these processing conditions. Although organic antimicrobial agents (essential oils) are used in very small quantity, most consumers do not prefer the odor of essential

oils in seafood packaging. Organic antimicrobial agents are often complex toxic bactericides that can leach from the polymer, causing health concerns. Organic antibiotic agents are also often heat-labile and readily degraded by humidity and mechanical processing, making organic antibiotic agents difficult to incorporate into the standard resin processing systems. Microbial antimicrobial resistance continues to be a concern with seafood packaging. Therefore, the use of inorganic antimicrobial agents is a wide area of research for seafood packaging. ChronoFlex antimicrobial polymers containing silver ions (Ag^+) exhibit a wide spectrum of antimicrobial activity, safety, and heat stability (Guggenbichler et al. 1999) (Garey and Reed 2010). As inorganic antimicrobial agents have a broad spectrum against several pathogens, they are increasingly incorporated into food packaging. The most extensively studied inorganic nanoparticles for antimicrobial packaging include titanium dioxide (TiO_2), zinc oxide (ZnO), magnesium oxide (MgO), and silver zeolite.

Singh et al. (2015) found that silver zeolite, AgSiO_2 , has good antimicrobial activity for *P. aeruginosa*, *S. putrefaciens*, *L. monocytogenes*, except for *Clostridium perfringens*. ZnAg has also shown good antimicrobial properties against *P. aeruginosa*, *S. putrefaciens*, and *L. monocytogenes* except *Clostridium perfringens*. Only ZnAg is capable to reduce the count of *C. perfringens* compared to silver zeolite and AgSiO_2 . Two mechanisms are proposed for the bactericidal action of silver zeolite. One is the action of silver ion itself released from zeolite and the other is that of reactive oxygen species generated from silver in the matrix. While oxygen has been reported to be necessary for the bactericidal activity of silver zeolite by some researchers, silver zeolite has also been reported to be effective on oral bacteria under anaerobic conditions by Matsumura et al. (2003).

TiO_2 is non-toxic and approved by the FDA for use in foods, drugs, and food contact materials. Recently, Bodaghi et al. (2013) developed a TiO_2 -LDPE film using a blown film extruder, which was tested on *Pseudomonas* spp. using a modified in-vitro test method. we found that the number of *Pseudomonas* spp. decreased significantly by 4 and 1.35 log CFU/mL after 3 h of ultraviolet A illumination on TiO_2 film and blank film, respectively. Hamilton-Brehm (2009) demonstrated that Roussin's black salt [$\text{Fe}_4\text{S}_3(\text{NO})_7$] at a concentration of only 2 μM , compared to 0.5 μM , inhibited the growth of *C. perfringens* vegetative cells, 3 μM for *L. monocytogenes*, and 1.3 μM for *Clostridium sporogenes* (5). Lysis of *Pyrococcus furiosus* cells by Roussin's black salt occurs both near the optimum growth temperature, that is, 98 °C, as well as at 4 °C, where the metabolic and enzymatic activities are effectively zero.

Arfat et al. (2015) researched the antimicrobial effect of fish protein isolate (FPI)/fish skin gelatin (FSG) films incorporated with 3 % ZnO nanoparticles (ZnONP , w/w, based on protein content) and 100 % basil leaf essential

oil (BEO) and observed that the shelf life of sea bass with the FPI/FSG-ZnONP-BEO film extended the shelf life of sea bass slices up to 12 days at refrigerated temperatures (Fig. 3), which was 8 days longer than the control. In ZnO nanoparticles, the primary cause of antibacterial function might be the disruption of cell membrane activity. ZnO nanoparticles exerted a bactericidal, not bacteriostatic, effect on *Campylobacter jejuni* by showing no recovery of the treated cells on drug-free MH plates as well as by rapid killing of 10^8 CFU/mL of freshly grown cells of three different *C. jejuni* strains (Xie et al. 2011).

Effect of antimicrobial agent on peroxide value and acid value

Peroxide value (PV) and acid value (AV) were chosen as useful indices to control food safety and quality in seafood, and the standard values of PV and AV were set at no more than 30 mequiv/kg and 3, respectively. These values were chosen because they indicate the initial stage of fat deterioration in fish and seafood. During the storage of seafood, PV increases very rapidly. Overview of studies testing organic antimicrobial and components in the Chemical Quality Indices of seafood are given in Table 3. Pezeshk et al. (2011), who showed that shallot and turmeric extracts were effective in retarding the production of primary lipid oxidation products in rainbow trout fillets stored at 4 ± 1 °C, found that sodium acetate, sodium lactate, and sodium citrate worked well in refrigerated sliced salmon. Lipid oxidation, expressed by PV and thiobarbituric acid (TBA) value, was delayed in samples treated with sodium acetate and sodium citrate. The TBARS value is considered as an index for malondialdehyde (MDA) content, the

most predominant products of secondary lipid oxidation; therefore, it is considered a good chemical indicator for quality assurance. According to Gomes et al. (2003), the maximum level of TBARS indicating good quality of fish is 1–2 mg MDA equivalents/kg of tissue. In this study, TBARS for the control, shallot (1.5 %), shallot (3.0 %), ajwain (1.5 %), and ajwain (3.0 %) treatment groups were lower than the proposed limits until days 6, 9, 12, 9, and 15, respectively (Sallam 2007). A solution of tea polyphenol (0.2 %, w/v) and rosemary (0.2 %, w/v) was used for dip pretreatment, and chitosan (1.5 %, w/v) was used for the coating. The results showed that chitosan coating was effective in retarding PV in large yellow croaker during the refrigerated storage (Li et al. 2012).

Effect of freezing techniques, high pressure, and electron beam on antimicrobial efficiency

Freezing, frozen storage, and thawing effect the quality and shelf stability of fish and seafood. If kept under appropriate condition, fish and seafood can be stored in the frozen state for several months without appreciable changes in quality. Application of the hurdle technology concept has been proposed as an approach to increase the microbicide effect of lower pressure processes. The combination of freezing, high pressure processing, and electron beam improves the effect of antimicrobial components. Burt (2004) concluded in a review that physical conditions that improve the action of EOs are low pH, low temperature, and low oxygen levels. Thymol and carvacrol exert a synergistic effect with high hydrostatic pressure (HHP). The viable numbers of mid-exponential phase *L. monocytogenes*

Fig. 3 *Pseudomonas* counts from sea bass slices wrapped without and with films (PP film, FPI/FSG film, FPI/FSGZnONP film, FPI/FSG-BEO film, and FPI/FSG-ZnONP-BEO film) during storage at 4 °C for 12 days. Source: Adapted and modified from Arfat et al. 2015

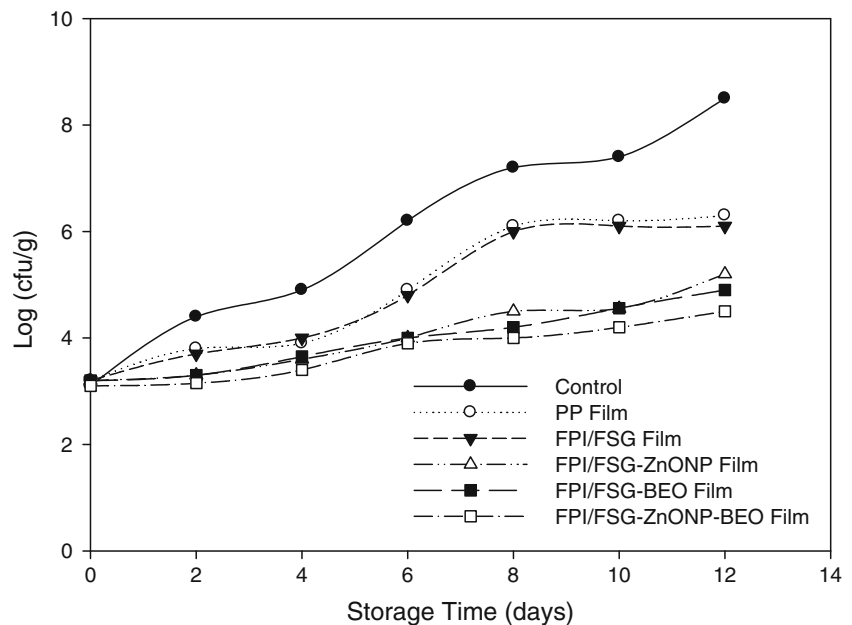


Table 3 Overview of studies testing organic antimicrobial agents and their components in the Chemical Quality Indices of seafood

Seafood	Organic antimicrobial	Concentration	Chemical Quality Indices	Reduction in final amount	References
Rainbow trout	Shallot fruit and ajwain seed	3 % v/w	PV TVB-N	++ ++	Raeisi et al. 2015
Jumbo squid	Phosphates, erythorbate	0.5 % v/w 0.15 % v/w	PV TVB-N	+++ +++	Ramírez-Suárez et al. 2015
Rainbow trout fillet	Oregano	0.4 % v/w	PV TVB-N	+ +	Mexis et al. 2009
Bighead carp fillets	Sodium alginate	1 % v/w	PV TBA	++ ++	Heydari et al. 2015
Turbot fillets	Essential oils	4 µL/L	(TVB-N) TBA TMA-N PV	+ + + +	Cai et al. 2015
Rainbow trout fillet	Cumin seed and wild mint leaf extracts	6.0 % v/w	TBARS PV TVB-N TMA-N	++ ++ + ++	Jalali et al. 2016
Silver carp fillet	Clove essential oil	1.5 % v/w	PV TVB-N	+ +	Jalali et al. 2016
Megrim	lyophilized alga, <i>Fucus spiralis</i> , and sorbic acid	8 % v/w 1 % v/w	PV, TMA-N	++ ++	García-Soto et al. 2015
Ovate pompano	Chitosan coating Citric acid Licorice extract	1.5 % w/v 0.6 % w/v 1 % w/v	PV TBARS	++ ++	Qiu et al. 2015

PV peroxide value, TVB-N total volatile base nitrogen, TBA thiobarbituric acid, TMA-N trimethylamine

++ high reduction compared to control, + medium reduction compared to control

cells were reduced more by combined treatment with 300 MPa HHP and 3 mmol/L thymol or carvacrol than by separate treatments. Gao and Ju (2008) studied the combined effects of pressure (300.0–700.0 MPa), temperature (30–70 °C), and the presence of nisin (0–333 IU/mL) on the inactivation of *Clostridium botulinum* 33A spores at various pressure-holding times (7.5–17.5 min). Günlü et al. (2014) observed that the shelf life of rainbow trout fillets was 4 days in the HHP group, 8 days in the chitosan-based film (CFW) group, and 24 days in the HHP + CFW group, when compared to the control group. In conclusion, it was determined that treatment with high pressure and wrapping with CFW had protective effect, both chemically and microbiologically, and that the most effective protection was obtained when both methods were used together. Ouattara et al. (2001) found that gamma irradiation combined with edible antimicrobial coatings showed significant potential for inhibiting aerobic bacteria, including *Pseudomonas putida*, and as a result, the microbial shelf life of shrimp was extended by 5 days with gamma irradiation, and by more than 11 days with gamma irradiation combined with a protein-based coating containing thyme oil and *trans*-cinnamaldehyde. Das et al. (2015) concluded that a combination of high pressure and potassium sorbate

dip treatment (at acidic pH) was very useful in inactivating *L. monocytogenes* in Indian white prawns. Potassium sorbate (0.1 %) dip for 15 min coupled with treatment with 250 and 350 MPa pressure reduced the level of *L. monocytogenes* by 2.345 and 5.908 log CFU/g, respectively.

Controlled release packaging (CRP)

This is a new generation of packaging materials that can release active compounds at different controlled rates and enhance the quality and safety of many food items during extended storage. The basic concept is to use the package as a delivery system for active compounds such as antimicrobials, antioxidants, enzymes, flavors, and nutraceuticals. Controlled release of antimicrobial agents from films is a very important parameter to study the efficiency and effectiveness of antimicrobial films and coatings in seafood packaging. Rossi-Márquez et al. (2009) found that the release of antimicrobial agents from packaging films depends on many factors such as electrostatic interaction between the antimicrobial agent and polymer chains, ionic osmosis, and structural changes induced by the presence of the antimicrobial agent and the environmental condition. Diffusion of the antimicrobial agent

Table 4 Standard methods for testing the resistance of plastic material to microbial attack

Method	Description
IEC 68-2-10	Basic Environmental Testing Procedures
EN ISO 846	Plastics – Evaluation of the Action of Microorganisms
ASTM G21-90	Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi
ASTM G22-76	Standard Practice for Determining Resistance of Synthetic Polymers
ASTM E1428	Standard Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by <i>Streptovorticillium reticulum</i>
ASTM E2149	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions
ASTM E2180	Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agents in Polymeric or Hydrophobic Materials
ASTM E2471	Standard Test Method for Using Seeded Agar for the Screening Assessment of Antimicrobial Activity in Carpets
ISO 846	Plastics-Evaluation of the Action of Microorganisms
ISO 16869	Assessment of the Effectiveness of Fungistatic Compounds in Plastics Formulations
ISO 22196	Plastics – Measurement of Antibacterial Activity on Plastics Surfaces Minimal Inhibitory Concentration (MIC) Zone of Inhibition (Kirby Bauer)

is also affected by the type of food, hydrophilic characteristics, and storage conditions. A natural antimicrobial known as lysozyme that inhibits lactic acid bacteria is incorporated in polyvinyl alcohol (PVOH) films. The degree of crosslinking of PVOH films helps maintain the release rate of the antimicrobial agent, in order to exert effective inhibition (Buonocore et al. 2003). Antimicrobial packaging materials were obtained by incorporating lysozyme into cellulose acetate (CA) films. In order to achieve controlled release of lysozyme, the structure of the films was changed from highly asymmetric and porous to dense by modulating the composition of the initial casting solution. The highest release rate, soluble lysozyme activity, and antimicrobial activity were obtained with the film prepared from 5 % CA solution, including 1.5 % lysozyme (Gemili et al. 2009). The application of antimicrobial films allows the migration of the antimicrobial agent to the film surface and provides continuous antimicrobial cover to the seafood during the extended transportation time.

Applications

Antimicrobial packaging for seafood is a rapidly emerging technology. The need to package seafood in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient, and safe seafood products predicts a bright future for antimicrobial packaging. The shelf life of fresh seafood is short and this poses a substantial problem in product distribution (Ashie et al. 1996). The purpose of the antimicrobial agents is to extend the shelf life of the food and to ensure safety by

reducing the rate of growth of specific microorganisms by direct contact of the package with the surface of solid foods (e.g., meat, seafood, cheese, etc.) or with the bulk of liquids (e.g., milk or meat exudates). In addition, antimicrobial packaging materials should be self-sterilizing or sanitizing. Such antimicrobial packaging materials greatly reduce the potential for recontamination of processed products and simplify the treatment of materials in order to eliminate product contamination (Han 2003). The application of antimicrobial films might allow the migration of the antimicrobial agent to the film surface, enabling continued antimicrobial effect on the food surface during extended exposure. Direct addition of antimicrobials to seafood will result in immediate reduction of the bacterial population, but this technique may not account for the recovery of injured cells or the growth of cells that were not destroyed by this direct addition (Quintavalla and Vicini 2002). The direct addition of antimicrobial agents to seafood may also change the organoleptic properties.

The use of antimicrobial packaging materials in seafood packaging can minimize the microbial contamination of seafood product surfaces during storage, transportation, and handling. The main action of these films is based on the release of antimicrobial substances into the seafood products. Some of these agents can pose a safety risk to consumers if the release is not tightly controlled by some mechanism within the packaging material itself. An interesting innovation would be the use of polymers with surfaces that have been modified by electron irradiation or plasma treatment to generate antimicrobial activity without any transfer or migration of the substances to the food (Buonocore et al. 2003).

As the growth and death rates of bacteria will vary for each growth medium, it is necessary to determine how antimicrobial films will perform for every food product.

Effectiveness testing

To evaluate if the antimicrobial packaging has an effect on the microorganisms present in seafood, agar plate method, minimum inhibitory concentration (MIC) determination, and dynamic shake flask test have been used; these methods are similar to those used to evaluate antimicrobials alone. In Japan, a method referred to as the “Film Contact Method” is used as the standard to assess the ability of products containing antimicrobial agents to impart antimicrobial properties to the products (SIAA 1998). The method was developed for inorganic antimicrobials such as silver-substituted zeolite. It is appropriate for films and sheets and involves inoculation of bacteria on the test specimen, followed by incubation and enumeration of the cells under specified conditions. The intent is to determine the resistance of the plastic to microbial growth, but it may also serve to determine if polymers are “self-sterilizing.” As shown in Table 4, standard methods have been used for testing antimicrobial efficiency. MICs can indicate the antimicrobial strength of the polymer and allow the comparison of polymer antimicrobial activity to that of the antimicrobial alone (Velazquez 2015). The MIC is the lowest concentration of an antimicrobial in a polymer resulting in complete growth inhibition of a test microorganism.

In the agar plate test, antimicrobial films are placed on a solid agar medium containing the test microorganism, which is then incubated until visible growth is attained. A clear zone surrounding the film indicates antimicrobial diffusion from the film and subsequent growth inhibition (Fig. 4). The agar plate test method simulates the wrapping of food items and indicates the possibilities when antimicrobial films are exposed to the contaminated surfaces and the antimicrobial agent migrates from the film to the food (Appendini and Hotchkiss 2002).

Challenges using organic and inorganic antimicrobial agents

Many of the antimicrobial compounds studied are not permitted for food application, as they need to migrate into the food to be effective. Technical challenges exist in incorporating appropriate antimicrobial agents into packaging systems. Most studies cited in this review article have been carried out at a laboratory scale; very few studies have been conducted in a pilot plant and none was conducted at an industrial

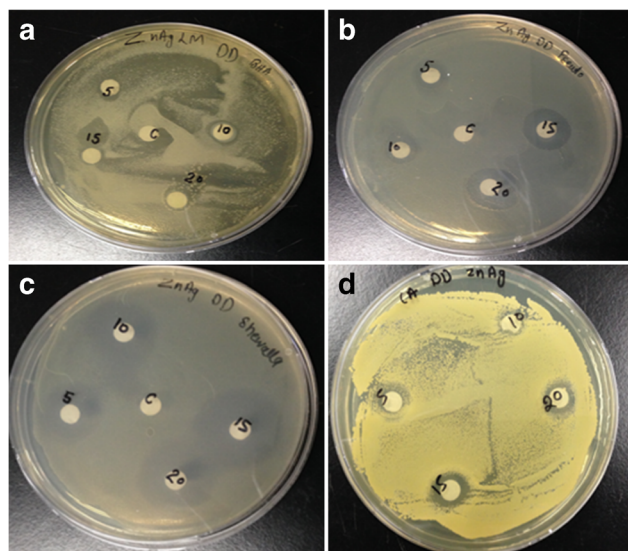


Fig. 4 ZnAg disk diffusion Inhibition zone for (a) *Listeria monocytogenes* (b) *Pseudomonas aeruginosa*, (c) *Shewanella gaetbuli*, (d) *Clostridium*

scale to ascertain the commercial value and scaling-up difficulties involved in this method.

Some of the antimicrobial agents like chitosan are still not permitted in the European countries; the major disadvantage of chitosan is that it is highly sensitive to humidity, and therefore, it is not suitable for seafood packaging. However, some researchers tried to solve this problem by designing water-resistant chitosan-based films. Current trends suggest that packaging will generally incorporate antimicrobial agents and that the sealing systems will continue to improve. The direct use of organic-based antimicrobial agents can have disadvantage based on their sensitivity to processing conditions. Many factors affect the application of antimicrobial agents on a commercial basis:

1. Synthesis of antimicrobial agents per their stability, simplicity, and cost effectiveness
2. The choice of an antimicrobial compound may be limited by not only its compatibility to the type of packaging material used, but also the heat resistance of the component during extrusion
3. Diffusion rate of the antimicrobial agents through the film
4. Physical properties of the packaging film, as affected by adding antimicrobial agents
5. Mode of action to inhibit bacterial growth
6. Interaction of antimicrobial agents within the food matrix
7. Resistance mechanisms
8. Toxicity release by antimicrobial agents

Researchers have primarily focused on developing new approaches and testing new methods on model systems, but not quite as much on real food products. Antimicrobial

packaging technology must focus on the technical feasibility, consumer acceptance, and food safety aspects of antimicrobial agents, in addition to their chemical, microbiological, and physiological effects. The final goal of antimicrobial seafood packaging to create a packaging film that does not harm human health and does not change the sensory or chemical properties of seafood and stable antimicrobial properties.

Future scope

Antimicrobial packaging is a novel development in the field of seafood safety; it plays a significant role in the future of “protection and preservation.” Organic-based antimicrobial agents are restricted in their activity because of harsh processing conditions during mixing with plastic. Seafood microorganisms easily develop resistance to organic antimicrobial agents; therefore, the continuous use of organic antimicrobial agents for seafood packaging is problematic. Future work will focus on the use of inorganic derived antimicrobial compounds (e.g., Ag^+ , Cu^{++} , Zn^{++}) that have a wide spectrum of activity and low toxicity. ChronoFlex antimicrobial polymers contain Ag^+ ions, which are preferred as they possess a wide spectrum of antimicrobial activity, are safe for human consumption, and are heat stable (Guggenbichler et al. 1999). ChronoFlex Antimicrobial Polymers can be processed by conventional extrusion and injection molding techniques while maintaining the desired antimicrobial properties. The silver-containing additive used in these Polymers is uniquely incorporated into the polymer structure during the polymerization sequence, thus ensuring uniform dispersion throughout the resulting polymer. The active silver ions incorporated in ChronoFlex Antimicrobial Polymers are stabilized by their association with carriers such as phosphates (in particular, zirconium phosphate), water-soluble silicate powder, zeolite, and ion exchange resin. It is possible that the development of “intelligent” or “smart” antimicrobial packages will follow. These materials will be able to sense the presence of microorganisms in the seafood, triggering an antimicrobial response in a controlled manner.

Antimicrobial packaging can play a significant role in reducing the risk of pathogen contamination, as well as extending the shelf life of seafood; however, it can never substitute for high-quality raw materials, properly processed foods, and good manufacturing practices. Antimicrobial packaging should be considered as a hurdle technology that, in addition to other non-thermal processes such as pulsed light, high pressure, and irradiation, can reduce the risk of pathogen contamination and extend the shelf life of perishable seafood products. Participation and collaboration of research institutions,

industry, and government regulatory agencies will be imperative for the success of antimicrobial packaging technology for seafood applications.

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