

Title	Biopolymer-based antimicrobial coatings for aquatic food products: A review
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Publication date	2022-02-05
Original Citation	Chakraborty, P., Nath, D., Hoque, M., Sarkar, P., Hati, S. and Mishra, B. K. (2022) 'Biopolymer-based antimicrobial coatings for aquatic food products: A review', Journal of Food Processing and Preservation, e16465. doi: 10.1111/jfpp.16465
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1111/jfpp.16465
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Download date	2025-04-21 07:25:06
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# Biopolymer-based antimicrobial coatings for aquatic food products: A Review

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the <u>Version of Record</u>. Please cite this article as <u>doi:</u> 10.1111/jfpp.16465

# Abstract

Aquatic food products, including fish and crustaceans, are some of the most consumed foods globally and are highly prone to microbial contamination. Such products have been preserved using conventional processing techniques such as freezing, cold storage, modified atmospheric packaging (MAP) and vacuum packaging. However, these techniques have been used since decades and are not cost-effective. Therefore, alternative sustainable strategies need to be explored. One viable option is the application of biopolymer-based films and coatings loaded with active antimicrobial agents (peptides and essential oil components) for the preservation of aquatic food products. Nisin is the most widely used peptide for the development of antimicrobial coatings, while eugenol, carvacrol, and cinnamaldehyde are among the most popular essential oil compounds. Findings reveal that both peptides and essential oils, when applied in combination within a coating system, demonstrate robust antimicrobial activity, delayed lipid oxidation, and retain the overall quality of the aquatic food system.

Keywords: active packaging; coatings; peptides; essential oils; aquatic foods

## Novelty impact statement

Antimicrobial-based coating systems have recently gained worldwide attention due to their ability to delay food contamination and maintain product quality throughout the storage period. This review provides a comprehensive description of peptide and essential oil-loaded coating systems and their application in the shelf life extension of aquatic food products. In addition, the application of nanocomposite systems for the preservation of aquatic foods have been discussed.

# 1. Introduction

Packaging is necessary to protect food products from various external factors such as light, moisture, mechanical shocks, dust, pressure, chemical changes, temperature, and ultraviolet radiation (Ribeiro-Santos, Andrade, de Melo, & Sanches-Silva, 2017). In addition, the contamination of foods by spoilage and pathogenic microorganisms (bacteria, yeasts, molds, and viruses) also poses a serious threat to food quality and safety (Liu, Sameen, Ahmed, Dai, & Qin, 2021). As per the Centre for Disease Control (CDC), the leading cause of foodborne infections includes Norovirus, nontyphoidal *Salmonella* spp., *Clostridium perfringens*, and *Campylobacter* spp. (Scallan et al., 2011). Most hospitalizations were caused due to nontyphoidal *Salmonella* spp., Norovirus, *Campylobacter* spp., and *Toxoplasma gondii* (Scallan et al., 2011). The major microbes responsible for human deaths included nontyphoidal *Salmonella*, *Toxoplasma gondii*, *Listeria monocytogenes*, and Norovirus (Scallan et al., 2011). In 2015, WHO reported that foodborne pathogens were responsible for causing serious infection to 60 million people, out of which 420,000 deaths occurred worldwide (WHO, 2015).

In addition to food safety issues, conventional packaging materials are non-biodegradable and possess a huge menace to the environment. The waste generated from the plastic packaging materials accounts for a considerable portion of solid waste leading to ever-increasing environmental distress (EPA, 2020). In 2018, an article published as "Plastic Pollution" by "Our World in Data" reported that 141 million tons of plastic waste were generated from packaging materials globally (Roser, 2018). Therefore, in order to improve food and environmental safety, the application of biodegradable polymers incorporated with active antimicrobial agents as food packaging materials appears to be a sustainable solution. The most important classes of sustainable polymers used in food packaging systems include carbohydrates and proteins (Dhumal & Sarkar, 2018; Hoque, Gupta, Santhosh, Syed, & Sarkar, 2021). Carbohydrates include different classes of molecules, which have found great significance in edible coating applications. These include starch (Resa, Gerschenson, & Jagus, 2016), cellulose and its derivatives (Roy & Rhim, 2020), alginates (Li, Zhou, et al., 2020), carrageenan (Roy, Ezati, & Rhim, 2021), and gums (Cao & Song, 2019; Li, Liu, Zhang, Meng, & Wang, 2021; Zhang, Zhao, & Shi, 2016). Similarly, proteins which have been utilized in edible films and coatings include a host of molecules such as whey proteins, caseins, soy proteins, kafirin, zein, and many others (Jiang et al., 2021; Olivera et al., 2019; Picchio et al., 2018; Tao, Sedman, & Ismail, 2021; Vilas Dhumal, Pal, & Sarkar, 2019). However, most biodegradable polymers do not exhibit native antimicrobial properties (Agarwal, Hoque, Bandara, Pal, & Sarkar, 2020; Dhumal, Ahmed, Bandara, & Sarkar, 2019; Llana-Ruiz-Cabello et al., 2016; Roy & Rhim, 2020), which makes the packaged product susceptible to microbial contamination. In order to impart antimicrobial properties to the packaging systems, different types of active antimicrobial agents can be incorporated to enhance the film functionality (Abdulmumeen, Risikat, & Sururah, 2012; Agarwal, Hoque, Mohapatra, et al., 2020). These active ingredients inhibit microbial growth significantly and delay the lipid oxidation of food products, ultimately leading to increased shelf life as these factors are the primary reason for food spoilage.

Fish products are extremely nutritious and receive great customer attention as a healthy animal protein source. During handling and storage, the quality of fresh fish deteriorates quickly limiting the shelf life of the product. High moisture content, neutral pH, high protein, and lipid contents make them an ideal candidate for spoilage. (Mohanty et al., 2016; Parlapani, Haroutounian, Nychas, & Boziaris, 2015; Ssepuuya, Mukisa, & Nakimbugwe, 2017). After death, endogenous enzymatic reactions initiated by native enzymes in fish muscle and viscera, such as proteases and lipases cause protein denaturation and lipid oxidation (Hussain et al., 2021). The end products of the above reactions, including biogenic amines (putrescine, histamine, and cadaverine) and off-flavor compounds, act as a nutrient medium for microbial growth (Hussain et al., 2021). Moreover, the production of such compounds leads to undesirable changes in sensory attributes and limits the shelf-life of fish products (Hussain et al., 2021). Similarly, crustaceans such as shrimp and prawns are highly perishable and prone to spoilage. The shelf life of these products is dependent on the extent of microbial population and enzymatic oxidation during storage (Alotaibi & Tahergorabi, 2018). Due to the presence of large amounts of free amino acids in shrimp, microbial growth occurs rapidly, which leads to spoilage (Alotaibi & Tahergorabi, 2018; Hussain et al., 2021). In addition, the unsaturated phospholipid contents undergo lipid oxidation and rancid off-flavor production even under refrigeration and frozen storage conditions (Alotaibi & Tahergorabi, 2018). To prevent the spoilage and quality degradation of these products, a lot of research has gone into using various preservation technologies to preserve or extend the shelf life of aquatic products, while assuring their safety (Albertos et al., 2017; Bilbao-Sainz et al., 2020; Bono et al., 2017). Among them, edible coatings loaded with bioactive antimicrobial compounds is a promising preservation method for aquatic food products since consumers nowadays prefer minimally processed safe foods, free from synthetic preservatives (Hussain et al., 2021).

The major active ingredients used in antimicrobial food packaging and edible coating systems consist of antimicrobial peptides and essential oils (Ju et al., 2019; Santhosh, Hoque, Sved, & Sarkar, 2021; Santos et al., 2018). The important antimicrobial peptides often used in bioactive food packaging include nisin-A (Santos et al., 2018), nisin-Z (Santos et al., 2018), pediocin (Espitia, Otoni, & Soares, 2016), and  $\varepsilon$ -poly-lysine (Liu, Liu, et al., 2020). These peptides can be incorporated into the packaging systems to fabricate antimicrobial films and coatings because of their exceptional antimicrobial efficacy, even at lower concentrations (Boelter & Brandelli, 2016; Meira, Zehetmeyer, Werner, & Brandelli, 2017; Phambu et al., 2017; Santiago-Silva et al., 2009). These antimicrobial peptides have been used in different types of formulations for the preparation of packaging films and coatings. For example, the shelf life of raw sliced pork was extended when pediocin was incorporated into polylactic acid/sawdust particle films (Woraprayote et al., 2013). The active biocomposite film was able to inhibit the growth of *Listeria* spp. by almost 99% on raw sliced pork, indicating its feasibility as a sustainable packaging material for high moisture food. Similarly, nisin incorporated polyhydroxybutyrate/polycaprolactone films enhanced the shelf life of ham as the growth of Lactobacillus plantarum was retarded due to the presence of nisin (Correa et al., 2017).

Essential oils have been the focal point of multiple types of research and are also utilized in synthesizing antimicrobial food packaging films because of their natural origin and non-toxicity (Agarwal, Hoque, Bandara, et al., 2020; Ahmed, Mulla, & Arfat, 2016; Dhumal et al., 2019). Essential oils are extracted from different parts of the plant, such as leaves, stems, roots, bark and are Generally Recognized As Safe (GRAS) by the US-FDA (Ribeiro-Santos et al., 2017). Some of the major essential oil components used in the preparation of food packaging materials include thymol (Othman, Nordin, Azman, Tawakkal, & Basha, 2021), eugenol (Cheng et al., 2019), carvacrol (Tao et al., 2021), cinnamaldehyde (Kardam, Kadam, & Dutt, 2021), limonene (Lan et

al., 2020), linalool (Das, Singh, Chaudhari, Dwivedy, & Dubey, 2021), geraniol (Agarwal, Hoque, Bandara, et al., 2020), and citral (Laorenza & Harnkarnsujarit, 2021). Essential oils have been reported to exhibit anti-inflammatory, anti-tumor, antidiabetic, antioxidant, and insecticidal properties (Ribeiro-Santos et al., 2017). In addition, these components also demonstrate potent antimicrobial activity against a broad spectrum of microbes, including bacteria (Agarwal, Hoque, Bandara, et al., 2020; Alizadeh-Sani, Rhim, Azizi-Lalabadi, Hemmati-Dinarvand, & Ehsani, 2020), yeasts, and molds (Kwon, Chang, & Han, 2017; Tariq et al., 2019). These components from the essential oils have been impregnated into the polymer matrix to develop antimicrobial packaging films for extending the storage period of food products. For example, da Rocha et al. (2018) in their study prepared agar films impregnated with clove essential oil for the shelf life extension of *Paralichthys orbignyanus* fillets. They reported that clove essential oil successfully inhibited the growth of foodborne pathogens such as Staphylococcus aureus, Yersinia enterocolitica, Aeromonas hydrophila, Debaryomyces hansenii, and Listeria innocua, which led to increased shelf life of fillet samples. Similarly, Alparslan and Baygar (2017) reported that chitosan films, when incorporated with orange peel essential oil improved the shelf life of deepwater pink shrimp by 8 days as compared to the control samples under refrigerated storage.

Even though these active compounds (both peptides and essential oils) demonstrate robust antimicrobial efficacy, they rapidly degrade when applied to real foods (Sarkar, Bhunia, & Yao, 2016b, 2017). For example, food systems contain a variety of proteolytic enzymes, which can degrade the antimicrobial peptides and reduce their efficacy (Sarkar et al., 2016b). Essential oils, when applied to food systems, are highly volatile in nature and can evaporate very quickly, leading to a loss in antimicrobial activity (Sarkar et al., 2017; Syed, Banerjee, & Sarkar, 2020). Therefore, in order to extend the efficacy of the antimicrobial compounds for a prolonged time, different strategies have been explored (Fu, Sarkar, Bhunia, & Yao, 2016). One of the viable options is to incorporate the antimicrobial compounds within biopolymer-based dispersions (Agarwal, Hoque, Bandara, et al., 2020; Fu et al., 2016; Roy & Rhim, 2021). The bioactive agents get impregnated into the polymeric matrix, which facilitates the sustained release of the active ingredients over a prolonged period of time. As a result, the films and coating exhibit antimicrobial activity for the entire storage period of the food product (Echeverría, López-Caballero, Gómez-Guillén, Mauri, & Montero, 2016; Faidi et al., 2019; Xu et al., 2018). These antimicrobial-loaded dispersions have been converted to films or coating systems (Fu et al., 2016). Therefore, this extensive state-of-the-art review aims to present the different types of antimicrobial peptides and essential oil components used in biopolymer-based antimicrobial food packaging systems with a focus on aquatic food products. Aquatic food products were selected as model systems since they are perishable food items and are highly susceptible to microbial contamination. The molecular structures of the active components, their chemistry, and the mechanism of antimicrobial action have been discussed in details. Thereafter, the application of the biodegradable packaging films incorporated with these active compounds on fish and crustacean products have been comprehensively reviewed. In addition, the application of nanocomposite materials in combination with antimicrobial agents used for coating of fish products have also been explored. Finally, the legal aspects of using peptides and essential oils have also been discussed.

#### 2. Chemistry of the antimicrobial compounds

#### 2.1 Antimicrobial peptides

#### 2.1.1 Nisin

Nisin is an antimicrobial peptide (bacteriocin) belonging to Class I bacteriocin, known as lantibiotics (Field et al., 2021; Santos et al., 2018) (Figure 1). Lantibiotics are characterized as small peptides having unusual amino acids such as  $\beta$ -methyl-lanthionine, lanthionine, and several dehydrated amino acids (Małaczewska & Kaczorek-Łukowska, 2021). Nisin is a cationic peptide consisting of 34 amino acids in the peptide chain containing four  $\beta$ -methyl-lanthionine and one lanthionine with a residual amount of dehydrobutyrine and dehydroalanine (Khan & Oh, 2016). Nisin is isolated from *Lactococcus lactis* and is regarded as the most prominent bacteriocin used as a food preservative for more than 60 years (Santos et al., 2018). There are two natural varieties of nisin, i.e., nisin A and nisin Z, having analogous antimicrobial spectra and structure but vary in amino acid position 27, where nisin A has histidine while nisin Z contains asparagine (Sarkar, Bhunia, & Yao, 2016a).

Nisin has been classified as Generally recognized as safe (GRAS) for its application as a food additive by the US-FDA due to its decomposition by digestive juices (Santos et al., 2018; Sarkar et al., 2016a). Nisin, when absorbed into the food matrix, does not lose its active properties even after sterilization, pasteurization, and other processing methods, making them highly desirable active ingredients (Santos et al., 2018). As a result, this bacteriocin has been widely incorporated into different biodegradable polymers to fabricate antimicrobial packaging materials (Gharsallaoui, Joly, Oulahal, & Degraeve, 2016). Different strategies have been used for

integrating nisin into food systems, such as emulsions (Sarkar et al., 2016b), liposomes (Lopes, Barreto Pinilla, & Brandelli, 2019), and solid particles (Liu, Sameen, et al., 2021).

# 2.1.2 ε-polylysine (EPL)

ε-polylysine (EPL) is a homopolymer, consisting of 25 to 30 lysine residues with linkages between the ε-amino group and α-carboxyl group (Figure 1) (Hinchliffe, Parassini Madappura, Syed Mohamed, & Roy, 2021; Wu, Sun, et al., 2019). It is also classified as a Dragendorffpositive substance, which is a quaternary nitrogen compound or an alkaloid (Shi, He, Feng, & Fu, 2015). It is a naturally occurring polypeptide produced from different strains of *Streptomyces albulus* as an extracellular compound (Wu, Sun, et al., 2019). EPL is a cationic peptide with high water solubility, biodegradability, edible, and nontoxic nature (Lin, Xue, Duraiarasan, & Haiying, 2018; Wu, Sun, et al., 2019). Moreover, EPL is thermally stable and does not show any inactivation or degradation even during autoclaving for 20 min (Shukla, Singh, Pandey, & Mishra, 2012).

EPL exhibits robust antimicrobial activity against both Gram-positive and Gram-negative bacteria along with phages and yeast (Shukla et al., 2012). However, the cationic nature of EPL might react with the anionic components present in the beverages and food matrix via ionic adsorption leading to the generation of unwanted precipitates, which weakens the antimicrobial activity and promotes food nutrient loss. Therefore, to circumvent these difficulties, researchers have developed antimicrobial delivery systems using anionic polysaccharides to incorporate cationic EPL (Lv et al., 2020).

#### 2.1.3 Antimicrobial mechanism of peptides

The mechanism of action of antimicrobial peptides (AMPs) such as nisin and  $\varepsilon$ -polylysine against microbial growth is still not completely well understood. A schematic mechanism of the action of antimicrobial peptides is illustrated in Figure 2 (Kumar, Kizhakkedathu, & Straus, 2018; Liu, Sameen, et al., 2021). Initially, these peptides were believed to result in pore formation and disintegration of microbial cell membranes (Tong, Ni, & Ling, 2014). However, recent studies have shown other possible mechanisms of antimicrobial peptides (Santos et al., 2018). The mechanism of action of AMPs starts with the strong electrostatic membrane interactions between the positively charged moieties of the peptide and negatively charged constituents on the outer surface of the bacterial cell (Moravej et al., 2018). This kind of electrostatic membrane interaction results in a strong bond formation between the positively and negatively-charged molecules,

which leads to the transport of the antimicrobial peptides through the bacterial cell. In Gramnegative bacteria, antimicrobial peptides demonstrate an uptake mechanism that is self-regulated. In this process, the peptides first replace the bivalent cations associated with the lipopolysaccharide (LPS), which leads to the destabilization of the cell membrane. On the other hand, in Gram-positive bacteria, the positively charged antimicrobial peptides get attached to the negatively charged teichoic acid molecules, which are found at the cell surface. These interactions lead to the invasion of the bacterial cell by the antimicrobial peptides (Moravej et al., 2018).

Antimicrobial peptides can also kill bacterial cells through invasive pathways like disrupting the bacterial cell membrane through pore formation, resulting in leakage of ions and metabolites. Cell death occurs due to the depolarization and loss of biopolymer synthesis. This invasion method involves several model systems. First is the barrel-stave model, where peptides having long chains penetrate the bacterial cell membrane forming a pore across the lipid bilayer (Moravej et al., 2018; Sekiya, Sakashita, Shimizu, Usui, & Kawano, 2018; Zhang et al., 2018). The second one is the toroidal-pore model, where the peptides are grouped and assembled within the membrane lipids and result in bending from top to bottom, which successively leads to membrane lysis and cell death (Moravej et al., 2018; Xu et al., 2020). The third model is the carpet model, where AMPs assemble themselves on the surface of the bacterial cell membrane like a carpet. This layer of AMPs then disrupts the order of lipids present on the bacterial cell membrane, which facilitates the entry of water molecules into the cell membrane. As a result, the cell membrane disrupts, and cell death occurs (Bogdanova, Valiullina, Faizullin, Kurbanov, & Ermakova, 2020; Li, Mei, & Xie, 2021). The fourth model is a detergent-like model where positively charged AMPs directly interact with the negatively charged cell membranes and form a strong parallel connection on the surface. As a result, nanopores are formed, and the cell membrane turns into nanomicelles. This results in cell membrane disintegration and leakage (Salas-Ambrosio, Tronnet, Verhaeghe, & Bonduelle, 2021).

## 2.2 Essential oil components

## 2.2.1 Eugenol

Eugenol (4-allyl-2-methoxyphenol), is a primary constituent (around 85%) of clove essential oil extracted through hydro-distillation of *Syzygium aromaticum* leaves and buds (Figure 1) (Burt, 2004; Ulanowska & Olas, 2021). It is an allyl chain substituted guaiacol that exhibits less acidity, low solubility in water, and is completely soluble in organic solvents. It is a pale yellow to

clear liquid which is the characteristic aroma of cloves with a pungent taste (Kamatou, Vermaak, & Viljoen, 2012). Eugenol has been used as a functional ingredient in pharmaceutical, food, cosmetics, and active packaging systems because of its robust antimicrobial and antioxidant properties (Marchese et al., 2017). It also exhibits anti-allergenic, anti-inflammatory, anti-swelling, anti-platelet, and analgesic properties apart from protection against carbon tetrachloride-induced hepatoxicity (Ulanowska & Olas, 2021). Moreover, eugenol is classified as a GRAS material by the US-FDA, making its incorporation safe for human consumption (Marchese et al., 2017). Encapsulation or incorporation of eugenol into different materials like films, emulsions, and nanomaterials is probably the best approach toward preserving its functional activity owing to its sensitive and volatile nature (Ulanowska & Olas, 2021).

#### 2.2.2 Cinnamaldehyde

Cinnamaldehyde (3-phenyl-2-propenal) is the biologically active constituent of the essential oil extracted from the genus *Cinnamomum* (about 85.3%), which gives cinnamon its characteristic taste and aroma (Figure 1) (Burt, 2004; Friedman, 2017). It is yellowish in color and extracted from cinnamon oil or produced synthetically by the condensation of acetaldehyde and benzaldehyde (Friedman, 2017). It can effectively retard the growth of foodborne pathogens (bacteria, yeast, molds) and is also classified as GRAS by the US-FDA (Kucinska-Lipka et al., 2019). Moreover, it also has various health benefits like anti-cancer, anti-inflammatory, and cardioprotection (Doyle & Stephens, 2019). Cinnamaldehyde exhibits high bactericidal activity against spoilage microorganisms and foodborne pathogens and acts as a natural preservative in retaining the quality of food products (Cao & Song, 2019). However, cinnamaldehyde is highly volatile and unstable, limiting its application in food biopreservation (Ji et al., 2019).

## 2.2.3 Carvacrol

Carvacrol (5-isopropyl-2-methylphenol) is a phenolic monoterpene compound consisting of phenolic rings with methyl and isopropyl substitutions (Figure 1) (Burt, 2004; Wang & Wu, 2021). It is the primary component present in oregano (*Origanum vulgare*), pepperwort (*Lepidium flavum*), thyme (*Thymus vulgaris*), and wild bergamot (*Citrus aurantium*) (Sharifi-Rad et al., 2018). The wide usage of this molecule is due to its numerous health benefits such as antimicrobial, antioxidant, anti-inflammatory, antimutagenic, anti-tumor, and insecticidal properties. The presence of the free hydroxyl group and the molecular configuration are primarily responsible for the strong antimicrobial activity of carvacrol (Wang & Wu, 2021). Furthermore, carvacrol has been classified as a GRAS material by the US-FDA, thereby increasing its application as an additive in the food and cosmetic industries (Ahmad, Elisha, van Vuuren, & Viljoen, 2021). Carvacrol has been used to fabricate antimicrobial packaging films by incorporating them into a biodegradable polymer matrix which resulted in the extended shelf life of food products (Tao et al., 2021).

#### 2.2.4 Linalool

Linalool (3,7-dimethyl-1,6-octadiene-3-ol) is acyclic monoterpenoid alcohol. an representing around 70% of the terpenoids of floral scents (Figure 1) (Burt, 2004; Das et al., 2021). Two different enantiomers of linalool are available, namely, S-(+)- linalool (coriandrol) and R-(--)-linalool (licareol), differing in their physiological and olfactory properties (Aprotosoaie, Hăncianu, Costache, & Miron, 2014; Das et al., 2021). Coriandrol possesses a floral, herbaceous, sweet, and petitgrain-like fruity and citrus notes, whereas licareol is perceived as a woody lavender-like aroma (Aprotosoaie et al., 2014). Linalool is available in the essential oils of approximately 200 different plant species, particularly from Coriandrum sativum, Lavandula angustifolia, Thymus vulgaris, and Cinnamomum camphora (Das et al., 2021). Linalool molecule has many beneficial properties like anti-cancer, anti-inflammatory, insect repellent, anxiolytic activity, along with robust antibacterial and antioxidant properties (Das et al., 2021).

#### 2.2.5 Thymol

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpene phenol which is a derivative of cymene ( $C_{10}H_{14}O$ ) and an isomer of carvacrol having a different position of -OH group corresponding to the methyl group (Figure 1) (Burt, 2004; Ezzat Abd El-Hack et al., 2016). It is the principal component of essential oil extracted from plants present in the *Lamiaceae* family, belonging to different genera such as *Ocinum, Origanum, Thymus, Thymbra, Monarda,* and *Satureja*; however, it is most commonly isolated from the *Thymus vulgaris* plant (Marchese et al., 2016). The biosynthesis of thymol occurs via aromatization of *p*-cymene from  $\gamma$ -terpinene followed by hydroxylation of *p*-cymene (Marchese et al., 2016). It is extracted as a white crystalline compound possessing a distinctive robust flavor of thyme herb with a pleasant aromatic smell (Ezzat Abd El-Hack et al., 2016). Thymol is a potent antiseptic compound and exhibits other beneficial properties such as anti-inflammatory, antioxidant, protection against oral diseases, and disorders affecting digestive and respiratory systems (Escobar, Pérez, Romanelli, & Blustein,

2020). But the application of thymol are not restricted to the pharmacological sector since it was classified as GRAS by the US-FDA as a food additive and preservative (Escobar et al., 2020). This development came due to the strong antibacterial activity exhibited by thymol against a wide spectrum of common foodborne pathogens like *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella* Typhimurium (Nabavi et al., 2015). Therefore, thymol is widely used as an active ingredient for formulating biodegradable packaging films capable of extending the shelf life of highly perishable food products (Loke, Chang, Hou, Cheng, & Hsieh, 2021; Othman et al., 2021).

## 2.2.6 Antimicrobial mechanism of essential oils

Essential oils are aromatic lipid compounds, largely present in flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots (Álvarez-Martínez, Barrajón-Catalán, Herranz-López, & Micol, 2021; Burt, 2004; Guo et al., 2021). Essential oils are classified into many groups, including terpenes, terpenoids, and phenylpropanoids based on their chemical structure (Álvarez-Martínez et al., 2021; Hussain et al., 2021). The chemical structure of individual essential oil plays a key role in their precise antibacterial activity. Essential oils containing aldehydes or phenols, namely, carvacrol, thymol, cinnamaldehyde, citral, and eugenol, possess the highest antimicrobial activity than terpene alcohol, ketones, or ester-containing essential oils (Dhifi, Bellili, Jazi, Bahloul, & Mnif, 2016). A schematic mechanism of the antimicrobial action of essential oil is shown in Figure 3 (Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013). The antimicrobial activities exhibited by essential oils mainly depend upon their composition of major and minor phenolic compounds (Álvarez-Martínez et al., 2021). The presence of different groups of chemical compounds in essential oil imparts several mechanisms on the target microorganism rather than one specific pathway (Dhifi et al., 2016). In general, the hydrophobic nature of essential oils and their components helps them to destroy the lipid architecture of the bacterial cell membranes, making them more permeable, which leads to the excessive leakage of intracellular ions and other components and finally leading to cell death (Álvarez-Martínez et al., 2021).

Phenolic compounds like carvacrol, eugenol, and thymol act in a somewhat similar fashion in the disruption of the bacterial cytoplasmic membrane. For example, the structurally similar carvacrol, and thymol, causes structural and functional damages to the cytoplasmic membrane (Marchese et al., 2016). Thymol forms a complex with membrane proteins through hydrogen bonds and hydrophobic interaction, which disrupts the outer and inner layer membrane, and increases the membrane permeability (Hyldgaard, Mygind, & Meyer, 2012; Marchese et al., 2016). In addition, thymol interferes in the energy-generating process, such as ATP synthesis, via impeding the citrate metabolic pathway and affecting the cell recovery ability after exposure (Marchese et al., 2016).

Carvacrol is known for its strong action against Gram-negative bacteria through the disintegration of the outer membrane and release of lipopolysaccharides (Ryu, McClements, Corradini, Yang, & McLandsborough, 2018). The interaction with the cytoplasmic membrane increases the fluidity and results in passive transport of ions across the membrane (Rathod, Kulawik, Ozogul, Regenstein, & Ozogul, 2021). Similarly, eugenol, a phenylpropanoid compound, interacts with membrane proteins, permeabilizes the cell membrane, and transports potassium and ATP out of the cell (Jeyakumar & Lawrence, 2021). The action of eugenol on the cell membrane occurs through a non-specific permeabilization mechanism (Hyldgaard et al., 2012). The hydroxyl group in eugenol could bind to the protein and impair its function, which affects the energy generation for cell recovery (Jeyakumar & Lawrence, 2021). The aldehyde groups in cinnamaldehyde can crosslink with the DNA and proteins through amine groups and impair their functions. Inhibition of enzymes that are involved in cytokinesis, acting as an ATPase inhibitor, and perturbing cell membrane are some of the modes of action of cinnamaldehyde against target microorganisms (Hyldgaard et al., 2012).

# 3. Application of antimicrobial peptide-loaded coatings for aquatic food products

#### 3.1 Fish products

Fish products have been majorly coated with two types of antimicrobial peptides, nisin and  $\epsilon$ -poly-lysine (EPL). The mechanism of coating of fish as a model food system has been demonstrated in Figure 4. Hui, Liu, Feng, Li, and Gao (2016) improved the storage quality of the yellow croaker fish (*Pseudosciaena crocea*) by coating it with chitosan and nisin at different concentrations. The active ingredient-treated samples exhibited higher sensory attributes, total viable counts (TVC), TVB-N, along with lower moisture loss, color degradation, and freshness quality index (K value). The preservative action of chitosan (1%) and nisin (0.6%) was deemed optimum as the samples coated with this formulation exhibited the best sensory and quality enhancement effects. Similar quality retention of northern snakeheads fish fillets was reported for alginate-calcium coatings incorporated with nisin, cinnamaldehyde, and EDTA (Lu, Ding, Ye, & Liu, 2010).

A study by Hager, Rawles, Xiong, Newman, and Webster (2019)), formulated a corn-zeinbased edible coating incorporated with lemongrass oil (8 %) and nisin (6 g). The authors coated fresh hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) to inhibit the growth of *Listeria monocytogenes* under frozen and refrigerated storage. The active ingredients loaded corn-zein coating reduced the bacterial population substantially. The log reduction was higher for nisin incorporated coating rather than the essential oil incorporated coating in both storage conditions, indicating a higher bactericidal effect of nisin. Moreover, the active coated products exhibited shelf life extension till 5 and 60 days for refrigerated and frozen storage conditions, respectively. Similar results were reported for cold-smoked sunshine bass coated with cornzein/nisin/lemongrass essential oil (Hager, Rawles, Xiong, Newman, Thompson, et al., 2019) and zein/nisin coated fish balls (Lin, Wang, & Weng, 2011).

Thermoplastic starch/polybutylene adipate terephthalate blend composite films coated with nisin Z and lauric arginate (LAE) for increasing the shelf life of bigeye snapper (*Lutjanus lineolatus*) was reported (Pattanayaiying, Sane, Photjanataree, & Cutter, 2019). The antimicrobial coated films were tested for the growth of *Salmonella* Typhimurium and *Vibrio parahaemolyticus* on the food samples during refrigerated and frozen storage. The antimicrobial coated films were able to decrease the *Salmonella* Typhimurium and *Vibrio parahaemolyticus* growth on sample products. Both the LAE coated and nisin/LAE coated films showed significant log reduction for the samples stored under refrigerated and freezing conditions, with nisin/LAE coatings exhibiting greater potency against the target microorganisms.

EPL in different concentrations (0.1 - 0.3%) was loaded into sodium alginate and chitosan to formulate active coatings for the quality retention of cultured pufferfish (*Takifugu obscurus*). The samples were packed under modified atmospheric packaging (MAP: 35% N<sub>2</sub>, 5% O<sub>2</sub>, and 60% CO<sub>2</sub>), vacuum pressure (VP), and air package (AP) for 18 days at 4 °C. The samples with EPL incorporated coating under MAP exhibited the best quality retention compared to VP and AP. The coated samples packaged within MAP had the lowest total viable count, lactic acid bacteria count, H<sub>2</sub>S producing bacteria, and *Pseudomonas* spp, along with the reduced formation of off-flavour compounds such as trimethylamine, TVB-N, and ATP related components. Moreover, the active coated samples substantially decreased the release of fish-like flavor compounds such as 2, 3-butanedione, octanal, (E)-2-octenal and 1-octen-3-ol, and hexanal. Therefore, the chitosan, sodium alginate, and EPL coating combined with MAP were successful in maintaining the quality parameters of pufferfish (Li, Zhou, et al., 2020).

Subsequently, Li, Mei, et al. (2021) applied the same formulation on obscure pufferfish (*Takifugu obscurus*) packed under MAP to prevent the myofibril degradation resulting in overall quality deterioration during cold storage for 18 days. The coating and MAP retarded the myofibril oxidation of the samples by keeping greater sulfhydryl concentration while impeding carbonyl group formation. Moreover, the active coated samples protected the myofibril Ca<sup>2+</sup>- ATPase activity and preserved the secondary and tertiary structures of the myofibril during cold storage. Japanese sea bass (*Lateolabrax japonicus*) was coated with alginate and EPL for their quality preservation during cold storage (4 °C) for 16 days (Cai, Cao, Bai, & Li, 2015). The samples coated with alginate and EPL blend exhibited the best results in terms of quality retention. The alginate/EPL coating maintained the pH, color, product hardness, acceptable K-value, and organoleptic properties of the sea bass as compared to the control sample. Moreover, the alginate/EPL coatings reduced the microbial counts for psychrophilic, mesophilic, lactic acid bacteria, and yeast, owing to the antimicrobial effect of EPL.

In addition to EPL, rosmarinic acid was added into the chitosan-based coating to extend the shelf life of half-smooth Tongue sole (*Cynoglossus semilaevis* Günther) fillets during refrigerated storage (Wu, Zhou, et al., 2019). The authors reported that the active coatings were successful in retarding the spoilage of the samples, with 0.1% EPL and 3 mg/L rosmarinic acid as the optimum formulation. The development of off-flavor components was significantly reduced. The K-value of the coated samples was also relatively low, while the myofibril degradation and deterioration of fillet microstructure were also retarded to a minimum due to the synergistic action of active ingredients. Furthermore, the coating of fillets reduced the generation of off-odour volatiles like hexanal, octanal, and 1-octen-3-ol during storage.

## 3.2 Crustaceans

Sodium alginate and chitosan loaded with nisin coatings were used to extend the shelf life of shrimp (*Penaeus vannamei*) under cold storage conditions by Cen et al. (2021)). The authors reported that the active coated shrimps exhibited lower total viable count (TVC), total volatile basic nitrogen (TVB-N), and freshness value than the uncoated samples. The samples coated with the active ingredients also achieved higher sensory scores than the control shrimps. Overall the shelf life and general acceptability of the active coated shrimps increased to 8 days with the inhibition of common foodborne pathogens such as *Vibrio* spp., *Psychrobacter* spp., *Carnobacterium* spp., and *Acinetobacter* spp. during storage. Thermoplastic starch/polybutylene adipate terephthalate film coated with gelatin antimicrobial solution containing nisin Z and LAE was prepared (Pattanayaiying et al., 2019). The antimicrobial efficacy of the prepared films was studied with the inoculated raw tiger prawn (*Penaeus monodon*) slices as model food under refrigerated and frozen storage conditions. The antimicrobial coating caused a 7 log CFU/g reduction of *Salmonella* Typhimurium and *Vibrio parahaemolyticus* after 14 and 28 days' storage at 4 °C, respectively. Similarly, the prawn slices stored at -20 °C storage showed 5.6 and 5.8 log CFU/g reduction of *S.* Typhimurium (after 7 days) and *V. parahaemolyticus* (after 14 days), respectively.

Similar to nisin, EPL-loaded chitosan coating on Pacific white shrimp (*Litopenaeus vannamei*) significantly inhibited the growth of mesophilic, psychrotrophic, and H<sub>2</sub>S producing bacteria during storage at 4 °C (Na, Kim, Jang, Park, & Oh, 2018). Modified Gompertz model was used to estimate the microbial growth in shrimps during storage. It was found that the lag time of the microorganism was delayed in the EPL/chitosan-coated shrimp compared to samples coated with EPL and chitosan alone. Therefore, the shelf-life of the coated shrimps was extended up to 9 days at 4 °C storage. Recent studies reporting the application of peptide incorporated antimicrobial films and coatings for aquatic food products have been provided in Table 1.

# 4. Application of essential oil-loaded coatings for aquatic food products4.1 Fish products

Hairtail fish (*Trichiurus haumela*) was coated with chitosan-eugenol nanoemulsion to increase its shelf life during storage at 4 °C (Liu, Shao, et al., 2021). They reported that chitosan-eugenol coating increased the shelf life of the sample up to 18 days in cold storage than the chitosan-alone coating (12 days). The eugenol incorporated coating displayed reduced pH, TBA, TVC, TVB-N, and electrical conductivity values as compared to chitosan coating, indicating a better preservative action. Moreover, the water holding capacity of the chitosan-eugenol coated samples also improved, which indicated a lesser extent of myosin denaturation in the fish muscle. Similarly, Li, Peng, Mei, and Xie (2020) formulated sodium alginate/flaxseed gum coating incorporated with eugenol (0.075-0.3% w/v) to extend the shelf life of Japanese sea bass (*Lateolabrax japonicus*) during chilled storage. The sodium alginate/flaxseed gum with 0.15% eugenol exhibited the best results and was able to decrease the production of off-flavour compounds like trimethylamine, TVB-N, and free amino acid build-up in the samples. Moreover, the eugenol-coated samples exhibited a significant decrease in the TVC, *Pseudomonas* spp, H<sub>2</sub>S

producing bacteria, psychrophilic counts, and formation of fishy flavor compounds (nonanal, hexanal, heptanal 1-octen-3-ol, 1-penten-3-ol, decanal, 2,3-pentanedione). The active coating was able to increase the shelf life to a maximum of 16 days with acceptable organoleptic scores. Chinese sea bass (*Lateolabrax maculatus*) also exhibited extended shelf life when the gelatin/ $\beta$ -cyclodextrin coating was incorporated with eugenol (0.15 and 0.30 %) (Zhou et al., 2019).

In another study by Zhou, Li, Fang, Mei, and Xie (2020)), the authors incorporated 1.5% (v/v) eugenol into the gelatin/ $\beta$ -cyclodextrin formulation. The formulation was coated on Chinese sea bass (*Lateolabrax maculatus*) and packed with modified atmospheric packaging (MAP) (60% CO<sub>2</sub>, 30% N<sub>2</sub>, 10% O<sub>2</sub>) followed by storage under super chilling conditions (-0.9 °C) for 36 days. The gelatin/ $\beta$ -cyclodextrin/eugenol active coating along with MAP was able to retard the protein denaturation, bacterial growth, and alkaline accumulation, TVB-N, TVC, and free amino acid content. The synergistic effect of MAP and eugenol-loaded coating extended the shelf life of the samples up to 30 days. This excellent preservative action was attributed to the lower O<sub>2</sub> concentration and higher CO<sub>2</sub> concentration, which inhibited the growth of spoilage microorganisms. Furthermore, the incorporation of eugenol prolonged the shelf life due to its broad spectrum of antimicrobial properties and antioxidant capacity.

Yang et al. (2016)) prepared EVOH films loaded with 3% (w/w) eugenol and applied on grass carp and stored under refrigerated conditions to extend their shelf life. The barrier properties of the developed film were decreased due to the absorption of moisture by the polymer chains, which resulted in decreased intermolecular attraction and accelerated moisture diffusion. The grass carp under active packaging exhibited lower TVC, TVB-N, TBA, and pH as compared to control packed samples, indicating the preservative ability of eugenol. Moreover, the active packaged fish samples demonstrated better texture indices than the control group, indicating the delay in the denaturation of myosin in the fish muscle and microbial growth. The EVOH/eugenol active packaging was able to increase the shelf life of the samples by up to 7-8 days.

EVOH films were fabricated with cinnamaldehyde 5% (w/w) and applied on snakehead fish (*Ophiocephalus argus*) to increase their shelf life at 4 °C storage conditions (Ma, Li, & Wang, 2017). The cinnamaldehyde incorporated films demonstrated higher tensile strength and oxygen permeability than pure EVOH films. This was due to the cross-linkage between the carbonyl group of cinnamaldehyde and the hydroxyl group of EVOH, which resulted in the formation of hydrogen bonding. The thermal stability of the active film also showed improved stability due to increased intermolecular cross-linkage density. Moreover, the active films also exhibited radical

scavenging activity and antimicrobial actions, which delayed the increase in pH, TVC, TVB-N, and water loss of the fish during storage. The cinnamaldehyde incorporated active films also extended the shelf life of the fish samples up to 14 days, which indicated the film's ability in shelf life extension of marine products.

Loke et al. (2021)) applied collagen or carboxymethyl cellulose/cinnamaldehyde coating over the plasma-treated LDPE films. The active packaging material was used to improve the shelf life of tilapia fish fillets (*Orechromis niloticus*) due to the antimicrobial and antioxidant properties of cinnamaldehyde. The collagen/cinnamaldehyde active coating yield better results than the carboxymethyl cellulose/cinnamaldehyde and LDPE films. The collagen coating with 6% cinnamaldehyde retarded the growth of *V. parahaemolyticus* and decreased the total plate count by 1.76 and 1.82 log CFU/g, respectively, when compared to the control samples. Nevertheless, all the active films delayed the generation of TVB-N and TBA during storage and extended the shelf life to 3 days, as well as maintained their sensory profile.

Cinnamon essential oil is also widely used as a preservative agent in food packaging materials, with cinnamaldehyde being its primary constituent. Carboxymethyl cellulose/chitosan blend coating was incorporated with cinnamon essential oil and glutaraldehyde (Valizadeh, Naseri, Babaei, & Hosseini, 2020). This active coating was applied on paper sheets to investigate their preservative actions on fish patties for up to 16 days at 4 °C. The cinnamon essential oil incorporated active paper sheets exhibited robust antimicrobial (TVC and total psychrophilic count) and antioxidant properties. Furthermore, the oil-loaded active sheets demonstrated a reduced value of pH, TBA, TVB-N, and free fatty acids than the control samples, which indicated the preservative action of cinnamon essential oil. The shelf life of the active packaged fish patties increased from 8 to 12 days, confirming their ability to retain and preserve the quality of marine food products. A similar result was obtained for cinnamon and grape essential oils-based oil-inwater nanoemulsion to improve the shelf life of chilled flathead mullet fillets (Ameur et al., 2021).

Chitosan films incorporated with carvacrol and grape seed extract microcapsules were developed to improve the shelf life of refrigerated salmon (*Salmo salar*) (Alves et al., 2018). The resulting active film exhibited higher moisture content, thickness, opacity along with gas and moisture barrier properties compared to control chitosan film. The decline in barrier properties was attributed to the presence of grape seed extracts, which reduced the crystallinity of the polymer interface. Furthermore, the grape seed extracts are hydrophilic and promote the bonding

between water molecules and hydroxyl groups, making the polymer more permeable. The samples packed with the active films demonstrated lower values of TVB-N and pH after seven days of storage than control and pristine chitosan packaged samples. The active films also reduced the growth of *Pseudomonas* spp, psychrophilic and mesophilic bacteria and contributed to the preservation of salmon for 4 -7 days during storage.

Chitosan/carvacrol coating was prepared to preserve the quality of tilapia (Oreochromis *niloticus*) fillets under iced storage conditions (Chaparro-Hernandez et al., 2015). The carvacrolloaded coating was most effective in retaining the texture and color parameters of the samples compared to other coating formulations in the study. Moreover, the growth of microbes such as total aerobic counts, total coliforms, V. parahaemolyticus, V. cholerae, and V. alginolyticus on the tilapia surface was hindered by almost 2 log CFU/g for the carvacrol-loaded coating. Flaxseed gum/sodium alginate films were incorporated with  $\beta$ -cyclodextrin and different concentrations of carvacrol (0.5 - 2.0 mL) to develop an active coating for the preservation of Chinese sea bass (Lateolabrax maculatus) (Fang et al., 2019). The mechanical properties of the films decreased as the carvacrol concentration increased, which was attributed to the development of structural deformity in the polymer matrix after carvacrol inclusion. The films with the highest carvacrol content also showed high antioxidant activity and inhibition of bacterial species such as V. parahaemolyticus, S. putrfaciens, S. aureus, and P. fluorescens. Moreover, the samples with carvacrol (1.0 mL and 2.0 mL) loaded coatings exhibited the lowest TVB-N, K-value, and microbial spoilage and were able to maintain the organoleptic properties of sea bass fillets till nine days of storage. A similar shelf life extension of refrigerated breaded hake medallions was reported for edible gelatin/carvacrol films (Neira, Agustinelli, Ruseckaite, & Martucci, 2019).

Choulitoudi et al. (2016)) extracted the essential oil from *Satureja thymbra* plants, with carvacrol being the major component in the oil. The authors incorporated the *Satureja thymbra* essential oil and extracts into the carboxymethyl cellulose matrix to develop an active coating for fresh gilthead seabream (*Sparus aurata*) fillets. The active coating exhibited moderate oxidative stress reduction and antimicrobial action against the TVC, *Pseudomonas* spp, and *Enterobacteriaceae* spp. The combination of essential oil and *Satureja thymbra* extracts increased the shelf life of the samples by 35%, while the same combination reduced peroxide value by three folds in the fillets during storage. In a similar kind of study, rosemary, thyme, and oregano essential oils (10% w/w) were incorporated into polylactic acid to develop films for minced fish

packaging. Linalool is present in abundant quantity in oregano and rosemary essential oil, as estimated by the authors. The developed active films exhibited lower mechanical and barrier properties as compared to control films primarily due to the plasticization effect of the essential oils, which probably disrupted the polymer network. Moreover, the oil-loaded films had a high antioxidant activity and reduced the oxidative stress on the fish samples, which was evident from the TBA test. The minced fish samples packed with active films retained their quality for four days with little foul odor (Zeid, Karabagias, Nassif, & Kontominas, 2019).

Shirazi thyme essential oil and clove essential oil blend were incorporated with farsi gumbased coating to improve the shelf life of refrigerated rainbow trout fillets (Dehghani, Hosseini, Golmakani, Majdinasab, & Esteghlal, 2018). The combination of essential oils effectively enhanced the quality parameters of fish fillets. The active coating significantly delayed the increase of TBARS, TVB-N, and peroxide values as compared to uncoated and pristine farsi gum coated fillet samples. The overall acceptability and sensory parameters of the active coated samples were significantly higher than the control samples. Moreover, the microbial growth (TVC, lactic acid bacteria, and psychrophilic count) on the sample was also significantly inhibited for the active coated samples. The synergistic effect of the essential oil combination was responsible for reducing the microbial count, retaining the quality of the fish fillets, and extending the shelf life up to six days.

Similarly, quince seed mucilage films were incorporated with different concentrations of oregano and thyme essential oil (1- 2%) (Jouki, Yazdi, Mortazavi, Koocheki, & Khazaei, 2014). The resulting active films were then used to wrap rainbow trout fillets during cold storage at 4 °C temperature to improve their shelf life. The active wrapped fillets had lower *Pseudomonas spp.*, *Enterobacteriaceae*, lactic acid bacteria, H<sub>2</sub>S-producing bacteria, along with lower aerobic and psychrotrophic count due to the high antimicrobial property of oregano and thyme essential oil. The lowest microbial count was observed for 2% thyme essential oil coated fillets. Additionally, 2% oregano essential oil had the lowest values of TBA, TVB-N, and trimethylamine, demonstrating the strong antioxidant activity of the oregano essential oil. The fillets wrapped with 2% thyme essential oil had the highest overall shelf life of 11 days as compared to other formulations and the unwrapped samples.

## 4.2 Crustaceans

Citral. and α-terpineol essential oil loaded poly(butylene adipate carvacrol, terephthalate)/poly(lactic acid) films were studied for active packaging of Pacific white shrimps (Laorenza & Harnkarnsujarit, 2021). The essential oils incorporation imparted the plasticization effect, which enhanced the compatibility among the polymers with a reduction in crystallinity and melting temperature. The oil-loaded films inhibited the growth of psychrotrophic bacteria and total viable counts within the maximum permissible limit up to nine days at 4 °C storage. The shrimp packed with carvacrol-based film showed the least lipid oxidation compared to other films. Interestingly, the essential oil inhibited the protein denaturation and pro-polyphenol oxidase formation in the packed shrimp during storage, which indicated the quality retention and decreased rate of deterioration during active packaging of shrimps.

Edible coating of shrimp with sweet potato starch and varying concentration of thyme essential oil (TEO) (2-6%) was studied by Alotaibi and Tahergorabi (2018)). The coating significantly inhibited the lipid oxidation and melanosis in the shrimps during storage at 4 °C up to eight days, which attributed to the strong free-radical scavenging activity of the essential oil. TEO at 2% coating showed 4 and 3 log CFU/g reduction in aerobic plate count at the end of storage compared to uncoated and starch-alone coated shrimps, respectively. TEO at 4 and 6% concentrations completely inhibited the growth of microbes throughout the storage.

In addition to oil incorporation into the coating or film-forming solution, the combination of two or more active components in formulation synergistically retards the spoilage with acceptable quality attributes. For example, kojic acid incorporation into chitosan and clove essential oil solution enhanced the antimicrobial efficacy in white shrimp coating (Liu, Zhang, et al., 2020). The chitosan/oil/kojic acid-coated shrimps showed 6, 3.5, and 1 log CFU/g reduction in total aerobic plate count after 15 days of storage at 4 °C compared to uncoated, chitosan-coated, chitosan/oil-coated shrimps, respectively. Moreover, the coating also effectively inhibited the color change, melanosis, moisture loss, changes in texture, and sensory properties of the white shrimp.

Thymol and purple potato extract films were incorporated into 2,2,6,6-tetramethylpiperidine-1oxyl radical (TEMPO) oxidized bacterial cellulose films to develop packaging materials for shrimps (Wen et al., 2021). The incorporation of active compounds resulted in the improvement of thermal stability, UV barrier, and water barrier activity of bacterial cellulose films, although the tensile strength declined. The bacterial cellulose films with active compounds also demonstrated high antimicrobial activity (against *E. coli, S. aureus,* and *L. monocytogenes*) and antioxidant activity due to the synergistic effect of thymol and purple potato extracts. Moreover, due to the high anthocyanin content in purple potato, the film also provides information regarding the total freshness of the packaged product. This is due to the ability of anthocyanin, which changes its color according to pH variation and makes it a freshness monitoring packaging system as well. Table 2 presents some of the recent studies where essential oil has been infused to the biopolymeric matrix to synthesize antimicrobial coatings for aquatic food products.

# 5. Application of nanocomposites on aquatic food products for shelf-life extension

A nanocomposite is a system where nanomaterials have been impregnated within biopolymer-based matrices for improvement in the material properties. Nanocomposite-based food packaging systems have been designed for the coating and shelf-life extension of aquatic food products. For example, in a study by Sayyari, Rabbani, Farahmandfar, Kenari, and Nadoushan (2021)), the authors developed gum-based active nano-coatings (basil seed gum and Lepidium perfoliatum seed gum) by incorporating Bunium persicum oil (BEO) at different concentrations (1-2% w/v). The active nano-coatings (ranging from 265.17 to 454.66 nm) were used for extending the shelf life of rainbow trout fillets (Oncorhynchus mykiss) over a period of 16 days at 4°C. The control fillet samples exhibited the highest peroxide value (PV) in the range of 0.90 to 6.50 meg/kg. The addition of BEO delayed the increment of PV with 2% BEO loaded gum coating exhibiting the lowest peroxide value (3.49 meq/kg) compared to the control at the end of the storage period. The TBA also demonstrated a similar trend, with the control sample having the highest TBA value ranging from 0.19 to 5.16 mg malonaldehyde/Kg while the 2% BEO loaded gum coating exhibited the lowest TBA value of 2.10 mg malonaldehyde/kg. Similarly, the increase in pH and TVB-N in the active coated value was also delayed due to the incorporation of BEO. The presence of several phenolic compounds in the BEO delayed the deterioration of the samples. The total viable count and total psychrotrophic counts in active nanocoated samples exhibited the lowest values due to the incorporation of BEO, which has robust antimicrobial activity and inhibited the growth of microorganisms. A similar shelf-life extension of Oncorhynchus mykiss fillets was observed when Foeniculum vulgare BEO was incorporated into basil seed gum and Lepidium perfoliatum seed gum-based coatings (Sayyari, Rabani, Farahmandfar, Esmaeilzadeh Kenari, & Mousavi Nadoshan, 2021).

Jafari, Jafarpour, and Safari (2017) developed chitosan/ rosemary essential oil (EO)-based nanocomposites coatings and coated *Huso Huso* fillets inoculated with *Listeria monocytogenes*.

The biochemical properties such as TVBN, PV, TBA, and pH reported lower values for the active coated samples as compared to the control samples due to the antioxidant properties of EO. Furthermore, the chitosan/rosemary EO coating was successfully able to inhibit the growth of L. monocytogenes from 4.14 to 2.23 Log CFU/ml after 16 days. This indicated the robust antilisterial effect of the coatings. The chitosan/rosemary EO coating also retarded the protein denaturation in fish samples until 8 days. Similarly, alginate/montmorillonite films loaded with marjoram EO was used to preserve the shelf life of L. monocytogenes inoculated rainbow trout slice (Alboofetileh, Rezaei, Hosseini, & Abdollahi, 2016). The authors reported that marjoram EO had a better anti-listerial effect than cinnamon and clove EO when the model food system was stored at 10 °C for 12 days. As a result, 1% marjoram EO enriched alginate/montmorillonite film was used to cover fresh rainbow trout fillets. The films covered with active nanocomposites exhibited lesser total viable count, L. monocytogenes, and psychrotrophic count. The L. monocytogenes counts after 15 days of storage was 6.23 Log CFU/g which was considerably lower compared to 7.38 Log CFU/g of the control samples. Moreover, the samples covered with marjoram loaded films had lower TBVN values compared to the control samples, which could be attributed to the inhibition of spoilage bacteria and the reduced capability of bacteria for deamination of non-protein nitrogen compounds.

Echeverría, López-Caballero, Gómez-Guillén, Mauri, and Montero (2018), prepared soy protein isolate/montmorillonite/clove EO based active nanocomposites for the shelf life extension of bluefin tuna (*Thunnus thynnus*) fillets. The fish fillets packed with active nanocomposites exhibited lesser log CFU/g values for total aerobic mesophiles, total viable bacteria, *Pseudomonas spp*, and *Enterobacteriaceae* counts as compared to the fillets packed with control film until 12 days. The authors concluded that the active nanocomposites have good antimicrobial action due to the slow release of clove EO from the film matrix resulting in the prolonged shelf life of packed samples. The lipid oxidation of the samples indicated by the TBARS value was 83% lesser in the active packed samples compared to the control samples. However, the color of active packed fillets was modified mildly due to the transfer of EO into the fish muscles. Nevertheless, there was no migration of montmorillonite particles into the fish matrix, which ensures its food packaging application.

Similarly, nanoparticles such as ZnO are also used to impart active properties to the films. ZnO and *Zataria multiflora* EO were incorporated into chitosan-based nanocomposites to develop active coatings for the shelf-life extension of Asian sea bass fillets (Lates calcarifer) (Mosavinia, Mousavi, Khodanazary, & Hosseini, 2021). The fish fillets were coated with the active nanocomposites and placed under refrigeration for 16 days. The active coated (chitosan/ZnO/EO) samples delayed the increase in free fatty acid, TVBN, TBA, and pH values of the samples till 16 days as compared to the control samples. The color attributes of the active coated samples were also deemed acceptable throughout the storage period. Furthermore, the sensory scores of the fish fillets were better than the control samples till 8 and 12 days for chitosan/ZnO and chitosan/ZnO/EO, respectively, indicating better preservative action of EO. Barani, Ahari, and Bazgir (2018) prepared LDPE films incorporated with Ag (1 - 5%) and TiO<sub>2</sub> for the shelf life extension of pikeperch fillets (Sander lucioperca). The authors reported that the films exhibited antimicrobial efficacy against E. coli, C. albicans, and A. niger due to the synergistic effect of both Ag and TiO<sub>2</sub> with the maximum inhibition of microbes observed at 3% Ag incorporated films. The films were used to wrap fresh fish fillets and kept under storage for 20 days at 4 °C. The films were successfully able to delay the growth of microorganisms as compared to the control samples. Similar improvement in the shelf life of silver carp fillets was observed when the samples were coated with chitosan/montmorillonite/rosemary essential oil (Abdollahi, Rezaei, & Farzi, 2014).

Arfat, Benjakul, Vongkamjan, Sumpavapol, and Yarnpakdee (2015) developed fish protein isolate (FPI)/fish skin gelatin (FSG) based nanocomposite films incorporated with ZnO and basil EO to improve the shelf life of sea bass slices. The FPI/FSG/ZnO/basil EO nanocomposite films had the highest inhibition of lactic acid bacteria, psychrophilic bacteria, H<sub>2</sub>S producing bacteria, *Pseudomonas spp*, and *Enterobacteriaceae* counts compared to the FPI/FSG/ZnO, FPI/FSG/ basil EO, polypropylene and unwrapped samples during the storage period of 12 days. Furthermore, FPI/FSG/ZnO/basil EO films delayed the increase in TVBN, PV, pH, and TBARS values. This has been attributed to the presence of nanoparticles and essential oil, which restricts the penetration of oxygen and moisture, leading to decreased lipid oxidation of samples. Moreover, the ability of the EO to scavenge reactive species like hydroxyl, superoxide, and peroxyl radicals probably helped in reducing the lipid oxidation in fish samples. The active wrapped fish samples maintained their quality for 12 days, while the control sample was only considered as fresh till 6 days. The addition of nanoparticles to improve the functionality of films is a convenient method, although it has some shortcomings. Efatian, Ahari, Shahbazzadeh, Nowruzi, and Yousefi (2021)),

in their study, prepared LDPE films incorporated with Ag-Cu and TiO<sub>2</sub> for maintaining the quality parameters of Nile Tilapia (*Oreochromis niloticus*). The authors reported that the Ag and Cu from the nanocomposite films migrated slightly to the fish sample (Ag <2.0  $\mu$ g/Kg and Cu <10  $\mu$ g/Kg), although it was lower than the allowed migration limit of 0.01 mg/Kg. Nevertheless, the nanocomposites films exhibited robust antimicrobial activities against *E. coli* and *L. monocytogenes* owing to the antimicrobial properties of Ag and Cu nanoparticles. The biochemical properties such as pH, free fatty acid profile and fat concentration of the tilapia samples wrapped with LDPE/Ag-Cu/TiO<sub>2</sub> films were the lowest compared to the control samples indicating that the packaging films could be successful for seafood packaging. The effect of nanocomposite-based coatings on aquatic food products have been presented in Table 3.

#### 6. Legal aspects of the use of essential oils (EOs) and antimicrobial peptides in food

EOs contain flavoring substances and for the EOs to be used in food or packaging materials that come in contact with the food products have to be registered by the European Commission (EC) (European Commission, 2008). Regulation (EC) No 1334/2008 issued by European Commission provides a list of definitions describing different types of flavorings and also contains the various requirement that must be fulfilled for their safe use. On 1<sup>st</sup> October 2012, EC adopted Annex I that contain the Union list of approved flavorings, and it is reviewed and updated periodically. The regulation restricts the addition of undesirable substances into food products that are not included in the Union list and sets maximum levels for certain substances. EOs are also approved by the United States Food and Drug Administration (FDA) and are Generally Recognised as Safe (GRAS) if they are used in recommended amount ((FDA), 2021). Although EOs are approved as food additives, they may cause allergic effects (Ribeiro-Santos et al., 2017).

The EOs having aldehyde or phenol groups are sensitive to eyes, skin, and mucous membrane and cause irritation due to allergic reactions (Sharma, Barkauskaite, Jaiswal, & Jaiswal, 2021). Some EOs such as lavender, clove, jasmine, sandalwood, rosewood, laurel, lemongrass, eucalyptus, and pomerance cause allergic effect (Ribeiro-Santos et al., 2017). In case of case acute oral ingestion, EOs may cause severe allergic effects (Tisserand & Young, 2014). As reported, ingestion of clove EO may cause acidosis, damage liver functioning, convulsion, ketonuria, or even coma. Citronella may cause poisoning that may result in vomiting, fever, convulsions,

cyanosis, and deep and rapid respiration (Sharma et al., 2021). Therefore, it is necessary to maintain the balance between the effective EO dosage and the risk of toxicity.

The regulation for the usage of antimicrobial peptides in food contact applications is very limited to date. Among others, nisin is one of the antimicrobial peptides approved for food contact application in the USA and EU. Nisin meets the FDA specifications (21CFR184.1538) and is considered to be GRAS ((FDA), 2021). EU classified nisin as E 234 and recognized it as a safe antimicrobial peptide for application in foodstuff ((EC), 2017). Similarly, ε-polylysine is also recognized as safe and in accordance to 21CFR170.30 ε-polylysine has been approved to be GRAS (FDA, 2003). However, antimicrobial peptides of this nature are studied extensively, but very few have been legally approved for their application in food. Therefore, more research towards their migration and biological activity *in vitro* and *in vivo* are required to improve the scientific foundation for developing their regulatory expects.

#### 7. Conclusion and future perspectives

This review outlines the recent studies undertaken to develop films and coatings enriched with active compounds to enhance the shelf life of aquatic food products. The degradation of food products by microbes is the primary cause of food spoilage. As a result, compounds capable of delaying microbial growth are required to be incorporated into the packaging systems to achieve food with higher quality and promote industry profitability. Natural additives such as antimicrobial peptides and essential oils are widely used as active ingredients in biodegradable food packaging systems because of their ability to retard microbial growth in packaged products. Fish products are highly perishable food items with quick spoilage under improper storage and favourable conditions. Therefore, the biodegradable films and coatings loaded with active ingredients like antimicrobial peptides and essential oils enhanced the shelf-life of fish products. The preservation action of the antimicrobial compounds may be influenced by numerous aspects like type of essential oil, food composition, the polymer used, storage conditions, time, and contact area. Antimicrobial films and coatings have huge potential in enhancing the shelf life of food products and eventually reducing or replacing the application of synthetic additives. Moreover, this can also mitigate the accumulation of non-biodegradable packaging wastes. Therefore, further research should focus on selecting suitable antimicrobial compounds, compatible polymer matrices, and a wide range of additional model fish products and crustaceans. Declaration

"No potential competing interest was reported by the authors."

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Figure 1: Chemical structures of Nisin A, EPL, and different essential oil compounds (Burt, 2004; Field et al., 2021; Hinchliffe et al., 2021)

Figure 2: Schematic diagram of the antimicrobial mechanism of peptides (Adapted from (Kumar et al., 2018; Liu, Sameen, et al., 2021))

Figure 3: Schematic diagram of the antimicrobial mechanism of essential oils (Adapted from (Nazzaro et al., 2013))

Figure 4: Schematic representation of fish coating systems using biopolymers and antimicrobial compounds

Coating compos	Coating composition		Storage	Targeted			
<b>Biopolymer</b> (s)	Antimicrobial peptides	aquatic food model	condition	microorganism	Antimicrobial activity	Reference	
Thermoplastic starch/polybutylene adipate terephthalate	Nisin Z, lauric arginate	Bigeye snapper slices, and Tiger prawn	4 °C for 28 days; -20 °C for 90 days	ays; -20 °C parahaemolyticus, and Salmonella spp. population are reduced by 7		(Pattanayaiying et al., 2019)	
Gelatin	Nisin, thymol	Rainbow trout fillets	4°C for 12 days	Total psychrophilic count, Lactic Acid Bacteria, H <sub>2</sub> S-producing bacteria count, <i>L.</i> <i>monocytogenes</i>	After the 12 days storage period, TVC reduced by 0.95 log CFU/g Total Psychrophilic bacteria reduced by 0.92 log CFU/g H <sub>2</sub> S producing bacterial count reduced by 0.66 log CFU/g Lactic acid bacteria count reduced by 0.95 log CFU/g <i>L. monocytogenes</i> count reduced by 0.65 log CFU/g	(Mohajer et al., 2021)	
Tartary buckwheat polysaccharide	Nisin	Tilapia fillets	4 °C for 12 days	TVC	After day 12, the TVC count experienced a log reduction of 1.1 log CFU/g <i>E. coli</i> and <i>S. aureus</i> population was inhibited by 54.01 and 94.49%, respectively	(Wang, Zhang, Jin, & Li, 2018)	
Chitosan	Nisin	Large yellow croaker	4 °C for 8 days	TVC	TVC was reduced by up to 2.7 log CFU/g after the end of the storage period	(Hui et al., 2016)	
Chitosan, sodium alginate	Nisin	Penaeus vannamei shrimp	cold storage	Psychrobacter, Vibrio, Acinetobacter and Carnobacterium	For untreated samples, the TVC crossed the acceptable value after 6 days while the treated samples were deemed acceptable till 14 days	(Cen et al., 2021)	

## Table 1: Peptide-loaded antimicrobial coatings for aquatic food products.

Coating compo	sition	Tested	Tested Storage Targeted				
<b>Biopolymer</b> (s)	Antimicrobial peptides	aquatic food model	Storage condition	microorganism	Antimicrobial activity	Reference	
Polyvinyl alcohol	Nisin	Rainbow trout fillet	4 °C for 12 days	Total Mesophilic Aerobic and Lactic acid bacteria	Total mesophilic aerobic bacteria reached 6 log CFU/g on the 4 <sup>th</sup> day of storage for the control samples, while the nisin treated samples reached the same level on the 12 <sup>th</sup> day	(Meral et al., 2019)	
Chitosan, ethylenediaminetetraacetic acid (EDTA)	Nisin	Fresh grouper fish fillet	4 °C or 25 °C	Mesophilic bacteria, psychrotrophic bacteria, coliforms, <i>Aeromonas,</i> <i>Pseudomonas,</i> and <i>Vibrio</i>	<ul> <li>Nisin/EDTA completely inhibited the growth of <i>E. coli</i> and <i>S. aureus</i> in nutrient broth</li> <li>Chitosan/nisin/EDTA films reduced <i>E. coli</i> and <i>S. aureus</i> population by 1.4 and 1.2 log CFU/cm<sup>2,</sup> respectively.</li> <li>Mesophilic bacteria and psychrotrophic bacteria count reduced by up to 1 and 2 log CFU/g.</li> </ul>	(Chang et al., 2021)	
Corn zein	Nisin	Hybrid sea bass (Morone chrysops × Morone saxatilis)	4 °C for 42 days	L. monocytogenes	<i>L. monocytogenes</i> count reduced by 3.5 and 3.7 log CFU/g for PVC and vacuum packaged samples	(Hager, Rawles, Xiong, Newman, Thompson, et al., 2019)	
Trout skin gelatin	Nisin	Rainbow trout fillet	4 and 10 °C for 30 days	L. monocytogenes	<i>L. monocytogenes</i> count was inhibited by nisin below the detectable limit (0.3 log CFU/g)	(Han, Tammineni, Ünlü, Rasco, & Nindo, 2013)	
Chitosan	Nisin, sodium lactate, sodium diacetate,	Cold- smoked salmon	20 °C for 10 days	L. monocytogenes	The treatment inhibited the growth of <i>L. monocytogenes</i> for at least 6 weeks	(Ye, Neetoo, & Chen, 2008)	

Coating comp	Coating composition		Storage	Targeted		
<b>Biopolymer</b> (s)	Antimicrobial peptides	aquatic food model	condition	microorganism	Antimicrobial activity	Reference
	potassium					
	sorbate,					
	sodium					
	benzoate					
* The reduction	on in bacterial coun	ts was determine	d with respect to	the control sample TVC. Tota	al viable count: TMC: Total mesophilic: PTC: Ps	vebrotrophie:

\* The reduction in bacterial counts was determined with respect to the control sample. TVC: Total viable count; TMC: Total mesophilic; PTC: Psychrotrophic; LAB: Lactic acid bacteria

## Table 2: Essential oils-loaded antimicrobial coatings for aquatic food products.

Coating composition		Tested aquatic	Storage condition	Storage condition		Reference
<b>Biopolymer</b> (s)	peptides	food model	Storage condition	microorganism	Antimici obiai activity	Kelerence
Alginate	Cinnamon oil and citrus extracts	<i>Merluccius</i> spp. fillets	4 °C for 28 days	Total viable count (TVC)	For Ozone treated samples, TVC reached 10 <sup>6</sup> CFU/ml in 21 days, whereas the control samples had a TVC value of 10 <sup>6</sup> CGU/ml in 7 days. For gamma-irradiated samples, no bacterial growth was observed even after 28 days.	(Shanka r, Danneel s, & Lacroix, 2019)
Alginate	Thyme, Oregano, and Pimento	Carp fillets	4 °C for 30 days	TVC, and <i>Pseudomonas</i> sp.,	Active coated samples showed acceptable TVC, pseudomonas spp count, <i>Enterobacteriaceae</i> count, and H <sub>2</sub> S producing bacterial count till 10 days	(Hao, Shah, Sterniša, Možina, & Mráz, 2022)
Chitosan	Artemisia dracunculus	Scomberoides commersonnianus fillets	4 °C for 16 days	Total mesophilic (TMC) bacteria and psychrotrophic (PTC) bacteria)	After 16 days of storage, total mesophilic bacteria and psychrotrophic bacteria count reduced by 1.5 and 1.8 log CFU/g	(Farsani pour, Khodan azary, & Hosseini , 2020)
Chitosan	Ferulago angulata	Rainbow trout fillet	4 °C for 16 days	Shewanella putrefaciens and Pseudomonas fluorescens	After 16 days of storage, the TVC and psychrotrophic bacteria exhibited a log reduction of 4.5 and 4.3 log CFU/g	(Shokri, Parastou ei, Taghdir, & Abbasza deh, 2020)
Chitosan	Clove	Tambaqui ( <i>Colossoma</i>	-18 °C for 120 days	cultivable psychrotrophic bacteria	Psychrotrophic bacteria population was completely inhibited after 120 days of storage under frozen conditions for the active coated samples	(Vieira et al., 2019)

Coating con Biopolymer (s)	nposition Antimicrobial peptides	Tested aquatic food model	Storage condition	Targeted microorganism	Antimicrobial activity	Reference
		<i>macropomum</i> ) fillets				
Chitosan, gelatin	Clove	Salmon fillet	4 °C for 15 days	Total viable count (TVC)	After 15 days of storage, the TVC reduced by 2.1 log CFU/ml	(Xiong, Kamboj, Ajlouni, & Fang, 2021)
Pectin	Clove	Bream (Megalobrama ambycephala)	4 °C for 15 days	Total viable count, Psychrophilic bacteria, Lactic acid bacteria, <i>Enterobacteriaceae</i> , <i>Pseudomonas</i> spp., H <sub>2</sub> S producing bacteria	After 15 days of storage the log reduction in microbial counts is given as follows: Total bacteria count: 3.8 log CFU/g Psychrotrophic bacteria: 2.3 log CFU/g <i>Enterobacteriaceae</i> : 2.5 log CFU/g <i>Pseudomonas</i> spp.: 3.4 log CFU/g H <sub>2</sub> S-producing bacteria: 2.3 log CFU/g	(Nisar et al., 2019)
Poly(butylene adipate terephthalate) and poly(lactic acid)	Carvacrol, citral, and α- terpineol	Pacific white shrimp	4 °C for 12 days	Total viable count (TVC) and psychotropic bacteria	The TVC and psychrotrophic bacteria counts reduced by 1.1 and 0.9 log CFU/g after 12 days of storage	(Laoren za & Harnkar nsujarit, 2021)
Chitosan/ carboxymethyl cellulose	Cinnamon oil, glutaraldehyde	Rainbow trout fish patties	4 °C for 16 days	Total viable count (TVC) and psychotropic bacteria	The TVC and psychrotrophic bacteria counts reduced by 2.18 and 0.6 log CFU/g after 16 days of storage.	(Valizad eh et al., 2020)
Gelatin	Eugenol	Chinese Seabass (Lateolabrax maculatus)	-0.9 °C for 30 days	Total viable count (TVC), H <sub>2</sub> S- producing bacteria, <i>Pseudomonas</i>	After 15 days of storage the log reduction in TVC, psychrophillic bacteria count, <i>Pseudomonas</i> count, and H <sub>2</sub> S-producing bacteria were 1.5, 1.2, 0.7, and 1.5 log CFU/ml respectively	(Zhou et al., 2019)

Coating composition		Tested aquatic		Targeted	
<b>Biopolymer</b> (s)	Antimicrobial peptides	food model	Storage condition	microorganism	Antimicrobial activity Reference
				spp. and	
				Psychrophilic counts	

\* The reduction in bacterial counts was determined with respect to the control sample. TVC: Total viable count; TMC: Total mesophilic; PTC: Psychrotrophic; LAB: Lactic acid bacteria

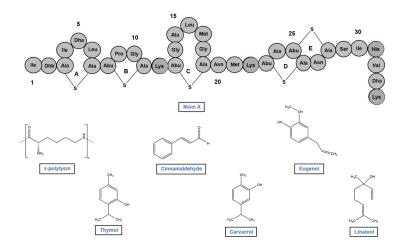
Table 3: Nanocomposite-based	antimicrobial	coatings for	<sup>,</sup> aquatic f	ood products.
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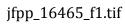
Coating composition		Tested aquatic	Storage	Targeted			
Biopolymer (s)	Nanoparticles	Antimicrobial peptides	food model	condition	microorganism	Antimicrobial activity	Reference
Fish protein isolate/Fish skin gelatin	ZnO	Basil leaf essential oil	Sea bass slices	4 °C for 12 days	Pseudomonas, H <sub>2</sub> S-producing bacteria and Enterobacteriaceae	On day 12, the total viable count, Psychrophilic bacterial count, Pseudomonas count, H <sub>2</sub> S-producing bacterial counts, Enterobacteriaceae counts and Lactic acid bacteria exhibited 3.1, 3.2, 2.9, 2.8, 2.7, and 2.5 log reduction, respectively	(Arfat et al., 2015)
Chitosan	Chitosan	Rosemary extract	Huso huso fillet	4 °C for 16 days	L. monocytogenes	On 16 <sup>th</sup> day of storage population of <i>L. monocytogenes</i> reduced from 4.1 to 2.23 log CFU/g	(Jafari et al., 2017)
Soy protein isolate	Montmorillonite	Clove essential oil	Bluefin tuna ( <i>Thunnus</i> <i>thynnus</i> ) fillets	2 °C for 17 days	TVC, Total aerobic mesophiles, H <sub>2</sub> S- producing bacteria, Luminescent colonies, <i>Pseudomonas</i> spp., <i>Enterobacteriaceae</i>	After 15 days of storage, the log reduction in microbial counts is given as follows: TVC: 2.1 log CFU/g Total aerobic mesophiles: 2.0 log CFU/g H <sub>2</sub> S-producing bacteria: <1 log CFU/g Luminescent colonies: <1 log CFU/g <i>Pseudomonas</i> spp.: 2.3 log CFU/g <i>Enterobacteriaceae</i> : >1.4 log CFU/g	(Echeverría et al., 2018)
Sodium alginate	Montmorillonite Clay	Marjoram essential oil (MEO) 1.5%	Rainbow trout slice	10 °C for 12 days	L. monocytogenes	After 15 days of storage period, <i>L. monocytogenes</i> , TVC and psychrotrophic count exhibited a log reduction of 1.15, 2.03 and 1.82 log CFU/g, respectively	(Zhou et al., 2019)
Chitosan	Montmorillonite Clay	Rosemary essential oil	Silver carp fillets	4 °C for 16 days	TVC, and TPC	The TVC and psychrotrophic count of the samples reduced by more than 1.5 log over the 16 day storage period	(Abdollahi et al., 2014)
Chitosan	ZnO	Zataria multiflora essential oil	Asian sea bass (Lates calcarifer)	4 °C for 16 days	Total mesophilic bacteria, TPC, and LAB	After 16 days of storage period, log reduction for total mesophilic bacteria, total psychrophilic bacteria and Lactic acid bacteria were 1.8, 1.2, 0.8 log CFU/g respectively	(Mosavinia et al., 2021)

(	Coating composition		- Tested aquatic	Storage	rage Targeted		
Biopolymer (s)	Nanoparticles	Antimicrobial peptides	food model	condition	microorganism	Antimicrobial activity	Reference
Basil gum and Lepidium perfoliatum gum	Basil gum and Lepidium perfoliatum gum	Foeniculum vulgare Essential Oil (2%)	Oncorhynchus mykiss fish fillets	4 °C for 28 days	Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli	After 28 days of storage, the log reduction for <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> were 3.59, 3.12, 3.79 log CFU/g respectively	(Sayyari, Rabani, et al., 2021)
Chitosan	ZnO	<i>Mentha</i> spicata Essential Oil	Rainbow Trout Fillets	4 °C for 28 days	TVC, TPC, Pseudomonas spp., and Enterobacteriaceae	After 14 days of storage, TVC, <i>Pseudomonas</i> count, psychrotrophic bacteria count, and <i>Enterobacteriaceae</i> count reduced by 4.7, 4.9, 4.7, 3.7 log CFU/g respectively	(Shahbazi & Shavisi, 2018)

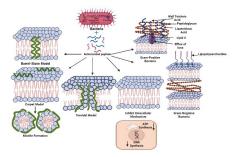
\* The reduction in bacterial counts was determined with respect to the control sample. TVC: Total viable count; TMC: Total mesophilic; PTC: Psychrotrophic; LAB: Lactic acid bacteria



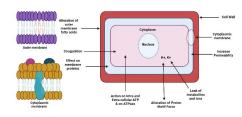




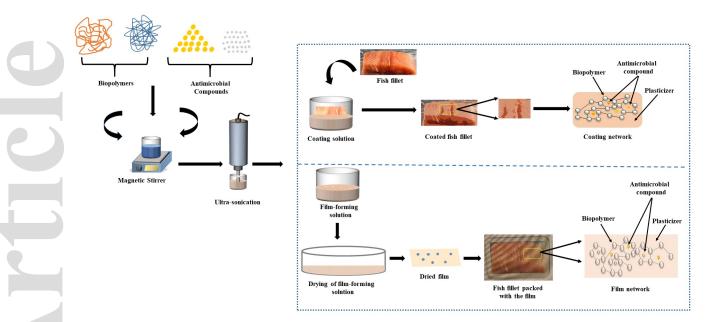




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