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Edible Films Incorporated with Active Compounds: Their Properties and Application

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Abstract

Antimicrobial compounds are food additives, which play a major role to reduce food spoilage. There are three main groups of antimicrobial compounds such as chemical agent, natural extract, and probiotics. The direct incorporations of the active compounds on the surface of food may have limited benefit because they are rapidly diffused from the food surface into the food product, resulting in the limited efficacy of these compounds. Thus, incorporation of antimicrobial compounds into packaging matrix, especially biopolymer film is a promising technique to reduce contaminations and inhibit, retard, and/or kill the microorganisms. Edible films are thin layer of natural polymers used to maintain the physicochemical quality of foods and extend their shelf life. A variety of biopolymeric-based materials including polysaccharides, proteins, and lipids have been extensively used for antimicrobial packaging and can be used as a carrier of active compounds. Incorporation of antimicrobial compounds may or may not enhance the mechanical properties and water vapor permeability of biopolymer films. The applications of active films can reduce contamination through the releasing of antimicrobial compound, thus reducing the risk from pathogen, extending shelf life of the packaged foods, and providing better quality with high safety.

Keywords: antimicrobial, edible film, bioactive compounds, natural extract, shelf life extension

1. Introduction

Antimicrobial compounds are functional additives, which play a major role to reduce food spoilage, maintain quality, and increase the shelf life of foodstuffs. There are three main groups of antimicrobial compounds such as chemical agent, natural extracts, and probiotics. Recently, consumers are increasingly seeking foods containing natural-occurring substances rather than synthetic additive because some synthetic additives can promote carcinogenic and toxicity, which is a clear concern to the health of consumers [1]. The use of antimicrobial compounds by directly adding to food products may cause the reduction of active compounds' activity and may change the organoleptic properties of the foods due to the complexity of food components and strong flavor of some agents. Thus, the incorporation of antimicrobial compounds into packaging matrix is a promising technique that can increase antimicrobial efficiency and solve the limitation of directly adding.

A variety of biopolymeric-based materials including polysaccharides, proteins, and lipids have been frequently used as packaging materials. Currently, many researchers have focused on the inclusion of natural extracts such as catechin, lysozyme, *Careya sphaerica* Roxb., the extract of longan seed, coconut husk, and lemongrass essential oils in biopolymeric-based edible films [1–5]. Edible films exhibit various advantages such as edibility, non-toxicity, biodegradability, biocompatibility, and barrier properties against oxygen and physical stress [1, 2, 4, 5]. In this context, they serve as a carrier of antimicrobial substances to prevent the microbial spoilage of foods.

The qualities of foods, including appearance, color, and texture, are important attributes; those influence consumers' perception and satisfaction. Most of the foods are highly perishable due to their food components, which make them prone to microbial deterioration. In addition, the growth of microorganisms can accelerate food deterioration leading to undesirable flavor and odor, discoloration, and change in texture. Therefore, the application of antimicrobial biopolymer film may allow the release of antimicrobial agents with continued antimicrobial effect on the surface of foods during controlled condition storage. This chapter will review the recent research on the properties of antimicrobial biopolymer films and its application on various types of foodstuffs.

2. Antimicrobial compounds incorporated in the edible films

Antimicrobial compounds are additives used to control biological deterioration and to inhibit the growth of microorganisms, including pathogenic microorganisms. There are several groups of antimicrobial compounds potentially incorporated into edible films, including chemical agents, natural extracts, and probiotics. Most of the antimicrobial compounds are classified as generally recognized as safe (GRAS). Recently, the consumer's desire for natural substances and for chemical preservative-free foods has been continuously increased.

A wide variety of antimicrobial substances could be incorporated into a packaging material to enhance antimicrobial property for controlling the growth rate of specific or groups of microorganisms in headspace of the package, ensuring food safety, and extending product shelf life. Many antimicrobial agents have shown strong antimicrobial activity against target microorganisms in the culture media. Nevertheless, many antimicrobial agents have limited effect on the microorganisms, used as food additives. The selection of antimicrobial compounds for food packaging materials should be based on the nature of active agents and their inhibition mechanisms, physicochemical characteristics of foods and the organoleptic property of active compounds, packaging manufacturing process and their effect on the efficiency of active agents, storage conditions, toxicity and regulatory issues, microflora of foods and the physiology of target microorganisms, and releasing mechanisms of active substances into foods [6]. Thereafter, antimicrobial compounds will be classified according to their nature.

2.1 Chemical agents

Organic acids and their salts are generally used as chemical antimicrobial agents for food products due to their efficacy and cost. They are produced by chemical synthesis or chemical modification of natural acids [7]. They showed antimicrobial effects on many types of microorganisms and had been potentially incorporated into edible films. The commonly used organic acids in film packaging are acetic acid, lactic acid, sorbic acid, citric acid, and their salts. Many studies have developed organic acid-contained biopolymer films and reported their antimicrobial

activity. For example, Uranga et al. [8] reported that 20% (w/w) citric acid-contained gelatin/chitosan films reduced *Escherichia coli* in liquid culture. da Rocha et al. [9] developed an anchovy protein films containing 1.50% (w/v) of sorbic acid or benzoic acid and evaluated antifungal effect of these films. The results suggested an effectiveness of antifungal films against *Aspergillus flavus* and *Rhizopus oryzae*. Organic acids can inhibit the growth of microorganisms by decreasing the pH, influencing the proton gradient cross membrane, acidifying the cytoplasm, and hindering transport chemicals across the cell membrane [10].

2.2 Natural extracts

2.2.1 Essential oils

Essential oils are aromatic and volatile oily extracts. Most of them are obtained from plant materials including leaves, flowers, roots, buds, and bark [11]. They can be utilized as flavoring in foodstuffs. However, the direct inclusion of essential oils as food preservatives is frequently limited owing to strong flavor. To avoid this problem, essential oils can be added into the edible films. The common used essential oils in bio-based film materials are cinnamon, clove, ginger, lemongrass, marjoram, oregano, sage, thyme, *Eucalyptus globulus*, and *Ziziphora clinopodioides*. They have shown to be against the various microorganisms [2, 12–15]. The antimicrobial activity of essential oils can be attributed to their major phenolic compounds such as thymol, eugenol, carvacrol, or terpenic compounds (α -pinene, β -pinene, 1,8-cineol, menthol, linalool), which are present in concentrations as much as 85% [11]. Different types of essential oils exhibited differences in their major compounds which had different ability to bind the membrane proteins of microbial cells and change the membrane permeability [16].

2.2.2 Plant and/or spice extracts

Recently, there has been an increasing interest in plants and/or spice extracts owing to the high levels of bioactive ingredients. Most of them are known to have a wide antimicrobial spectrum against microorganisms. The extracts can be obtained from various parts of plant and spice, including seeds, roots, bark, buds, flowers, and leaves. Antimicrobial efficiency of plant and/or spice extracts is generally attributed to the phenolic compounds that present in extracts. Phenolic compounds such as catechin, tannin, ferulic acid, caffeic acid, gallic acid, and carvacrol are present in parts of plants. Apart from antimicrobial efficiency, other benefits they offer include antioxidant capacity and their effect as alternative medicines. They are generally more effective against Gram-positive bacteria than that against Gram-negative bacteria due to the complex cell structure of Gram-negative bacteria.

2.2.3 Enzyme

The most employed as an antimicrobial enzyme is lysozyme. Lysozyme is a nutraceutical and is produced from egg white, milk, and blood [10]. It showed to be more effective against Gram-positive bacteria than Gram-negative bacteria by separating the bonds between N-acetylmuramic acid and N-acetylglucosamine of the peptidoglycan in the cell wall of bacteria [17]. Rawdkuen et al. [17] studied the antimicrobial effect of lysozyme on *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua*, and *Saccharomyces cerevisiae* and found that lysozyme could inhibit only the growth of *L. innocua* and *S. cerevisiae*. Furthermore, it has been reported that the combination of lysozyme with other substances can initiate membrane disruption. Rawdkuen et al. [17] found that the combination of lysozyme with catechin

in the ratio of 1:1 showed significance inhibited in all microorganisms tested, when compared with lysozyme alone. Branen and Davidson [18] reported that small amounts of ethylenediamine tetraacetic acid (EDTA) could enhance the activity of lysozyme against Gram-negative bacteria. The use of antimicrobial enzyme should be considered carefully because antimicrobial efficiency is highly sensitive to the substrates and the environments. For instance, lysozyme activity can be significantly influenced by pH and temperature.

2.2.4 Bacteriocins

Bacteriocins are naturally occurring antimicrobial substances. They are small molecular weight peptides produced by microorganisms and effectively inhibit the growth of food spoilage bacteria, mainly Gram-positive bacteria [10]. Many bacteriocins, including nisin, pediocin, and lactacin, can be incorporated into edible films to inhibit the growth of spoilage and pathogenic microorganisms. The most employed as antimicrobial bacteriocins is nisin. Nisin is an antimicrobial peptide which is produced by *Lactococcus lactis* ssp. *Lactis* and is known to exhibit antimicrobial activity against a wide range of Gram-positive bacteria. It has limited activity against Gram-negative bacteria because of its inability to penetrate the cell and reach to the cytoplasmic membrane [10]. Many studies have been reported that the combination of nisin with chelating agent (EDTA) improves the antimicrobial activity of nisin against Gram-negative bacteria by destabilizing the outer membrane and then releasing the lipopolysaccharide layer and allowing nisin to access the cytoplasmic membrane [19–21]. Antimicrobial efficiency of bacteriocins is influenced by its concentrations and number and species of microorganisms, using condition, interaction or inactivation by food elements, and temperature and pH of product [10].

2.3 Probiotics

Probiotics are live microorganisms that have a beneficial effect on health upon ingestion in sufficient numbers [22]. The most frequently used bacteria belong to the genera *Lactobacillus* and *Bifidobacterium*, although *Streptococcus thermophilus* and *Saccharomyces boulardii* are available in some dairy products. These naturally produced antimicrobial can inhibit the growth of strains of other bacteria. The use of probiotics can efficiently control the competitive undesirable microorganisms [7]. Recently, Bekhit et al. [23] reported that hydroxypropyl methylcellulose (HPMC) films containing microencapsulation of *Lactococcus lactis* subsp. *Lactis* showed effectiveness to reduce the growth of *L. monocytogenes* by five-log cycle after 12 days of storage, compared to the control film (without addition of *L. lactis*). Beristain-Bauza et al. [24] reported that whey protein isolate films containing cell-free supernatant of *L. rhamnosus* (12 or 18 mg/ml to film-forming solution) showed inhibitory activity against Gram-negative (*E. coli* and *Salmonella typhimurium*) and Gram-positive bacteria (*L. monocytogenes* and *S. aureus*). In addition, probiotics may be carried within edible polymer matrix used in the food packaging industry due to its safety and effectiveness.

3. Properties of biopolymer-based film

Antimicrobial film should be provided the same basic functions as the traditional food packaging. In general, the properties of edible film depend on type of raw material, film additive, concentration, process for making film, and condition used [25]. All of these factors must also be taken into account when making antimicrobial biopolymer-based film. When antimicrobial substance is incorporated

into edible film to retard the microbial growth, it can adversely change the original mechanical integrity, barrier properties, physical properties, and thermal stability of edible film. General properties of edible film include mechanical properties (tensile strength and elongation at break), barrier properties (water vapor permeability), and optical properties (color and transparency).

3.1 Mechanical properties

Mechanical properties are basic functions of food packaging which protect the food from physical damage such as denting, breaking, and bruising. The investigation of mechanical properties of antimicrobial film can provide the information of the film flexibility and the resistance of the film to the force. This information will be also used to predict the efficiency of antimicrobial film during handling, storage, or use. Mechanical properties are commonly expressed as tensile strength (TS) and elongation at break (EAB). TS represents the maximum strength that a film can withstand, while EAB is the measurement of the ability of a film to stretch. The mechanical properties of antimicrobial bio-based film are shown in **Table 1**.

Incorporation of antimicrobial compounds may or may not enhance the mechanical properties of edible films. It has been frequently reported that the mechanical properties of antimicrobial film are strongly dependent on concentration of antimicrobial compounds. Higher concentrations of antimicrobial agent are expected to lead to lower film strength and greater film extensibility because high amounts of antimicrobial compound inclusion may help to enhance the plasticizing effect of the edible film, as a consequence improving the film's extensibility [4, 5].

However, some researchers have reported that the inclusion of antimicrobial agent above a certain limit led to a decrease in the film stretch [1, 26]. Thus, prior to making antimicrobial film, the concentration of antimicrobial agent should be considered. Good mechanical properties are among the basic requirements for antimicrobial film to be used as active food packaging, since poor extensibility or strength may lead to premature failure or cracking during production, handling, storage, or use.

3.2 Barrier property

The barrier property extensively studied in food packaging material is water vapor permeability (WVP). WVP of packaging films is extremely the main function for preserving the quality or prolonging the shelf life of packaged food products. Generally, lower WVP values indicate decreasing film permeability, due to physiochemical deterioration and microbial spoilage that are related to the equilibrium moisture content. Thus, the film should have low WVP because the film is mainly used to avoid moisture transfer between the food and the environmental surrounding, as a result, maintaining quality and extending shelf life of packaged food products. However, the specific requirement for the films' WVP depends on characteristics of food products and intent use. **Table 1** summarizes data on WVP of various antimicrobial films.

It has been reported that the addition of antimicrobial agent into edible film could reduce the films' WVP has been reported. Antimicrobial films showed less WVP values than the films without addition of antimicrobial agents by about one–two times (**Table 1**). The dramatic decrease in the antimicrobial films' WVP is due to reduction in free volume space of polymer, decreasing an ability to bind with water molecules and increasing tortuosity of diffusive pathway through the film [1, 17, 27, 32]. However, antimicrobial substance-added edible films could also lead to higher in the films' WVP [3, 32]. It has been reported that antimicrobial agents could play

Based film	Antimicrobial compounds	Loading	Mechanical properties		WVP ($\times 10^{-10}$ g m/ m ² s Pa)	Refs.
			TS (MPa)	EAB (%)		
Basil seed gum	Oregano essential oil	1–6% (v/v)	—	—	0.37–0.43	[27]
Chitosan	Apple peel polyphenols	0, 0.25, 0.50, 0.75, and 1%	16–27	13–28	1.00–1.47	[28]
Chitosan	Curcumin	0–1% (w/w, based on chitosan)	6.64–11.82	79.13–39.41	2.24–2.69	[29]
Chitosan	Turmeric extract	1:2 (v/v, chitosan: extract)	32.2–47.9	6.20–6.35	1.12–1.53	[30]
Sodium alginate	Sage oil	0–1% (v/v)	4.8–5.2	4–78	1.90–2.36	[16]
Sodium alginate-carboxymethyl cellulose	Cinnamon essential oil	5, 10, and 15% (w/v)	16.07–32.10	20.07–32.67	13.66–28.01	[31]
Tapioca starch	Grape pomace extracts	8% (v/v)	2.58–3.55	22.5–45.8	13.42–20.22	[32]
Chicken feet protein	Marjoram oil	0–1% (w/v)	7.13–7.59	11.92–21.78	25.90–34.90	[14]
Fish myofibrillar protein	Catechin-Kradon extract	0–12 mg/ml	6.53–10.31	51.38–132.76	15.60–20.80	[1]
Gelatin	Catechin-lysozyme	0–0.5% (w/v)	3.31–33.49	27.82–143.87	650–1360	[4]
Gelatin	Longan seed extract	50–500 ppm	49.53–52.84	17.42–18.62	0.29–0.40	[5]
Gelatin	Coconut husk extract	0–0.4% (w/w)	34.97–41.93	6.16–7.90	0.23–0.28	[3]

Table 1. Mechanical properties and water vapor permeability (WVP) of antimicrobial films.

a role as a plasticizing agent in the films, leading to the interaction between intermolecular reduction and the enhancement of macromolecules' mobility [32]. As a consequence, the film had high WVP values. In addition, the films' WVP are affected by the chemical nature of macromolecules and additives, hydrophilic/hydrophobic ratios of the films, and the structural characteristic of macromolecules [32, 33].

3.3 Optical properties

Optical properties (color attributes and transparency) of food packaging are crucial parameters, which directly affect to the consumer acceptability and impact on product appearance. In general, color attributes are expressed as L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) values. Transparency

was used to evaluate film transparency wherein higher transparency value indicates lower film transparency. The optical properties of antimicrobial film packaging are summarized in **Table 2**. Recently, several researchers have reported that the addition of antimicrobial compounds could influence the optical properties of edible film by decreasing lightness and film transparency values and increasing redness and yellowness values correlated with the increasing content of antimicrobial agent [1, 5, 28].

Kaewprachu et al. [1] reported that the incorporation of catechin (obtained from commercial product)-Kradon extract (extracted from the leaves of *Careya sphaerica* Roxb.) could adversely affect the films' color, especially at higher amounts of catechin-Kradon extract. They concluded that the presence of natural pigment in catechin-Kradon extract can contribute to the color of fish myofibrillar protein film. Xu et al. [32] studied the effect of source of grape pomace extracts from Cabernet Franc (red variety) and Viognier (white variety) on the optical property of tapioca starch film. The results showed that source of extract had significant influence on film transparency. Inclusion of these extracts decreased transparency of films. Changes in films' color and transparency depend on source of active compounds, level of active agent incorporation, and original pigment present in active agent.

Therefore, film with low transparency may not be suitable for food packaging applications when the film is used as a see-through packaging material and used to enhance product's appearance. Although the color and transparency of antimicrobial film could affect the consumer perception, there are many advantages to helping to protect the packaged foods from ultraviolet and visible light that lead to discoloration, nutrient losses, and off-flavor and to showing antimicrobial properties that do not exist in the traditional packaging.

3.4 Antimicrobial property

The addition of antimicrobial compounds into edible films is expected to enhance their antimicrobial activity. Edible films can act as carriers of antimicrobial agents, which may either be immobilized into the film matrix or play their role upon contact with food surface or be slowly released into foodstuffs. The antimicrobial activity of films is commonly assessed through agar disc diffusion method, regarding the diffusion of substances tested through water-containing agar plate. The film cuts are placed on an agar surface which is previously inoculated with the test microorganisms, incubated under suitable conditions depending on the test microorganisms, and then observed the inhibition zone around the disc films [30]. The antimicrobial activity of antimicrobial agent-contained edible films was found against a wide variety of microorganisms, including bacteria and some fungi. The antimicrobial activity of active agents used in edible films against some microorganisms is presented in **Table 3**.

It has been frequently reported that inclusion of antimicrobial compounds has greatly enhanced antimicrobial property of edible films. Higher amounts of antimicrobial agent are expected to lead to higher antimicrobial effects. It should be noted that the antimicrobial efficiency of antimicrobial substances added into edible film can depend on the target microorganisms (Gram-positive bacteria, Gram-negative bacteria, fungi, and others), the type of antimicrobial agents, the level of antimicrobial agent incorporation, and their major compounds [14]. Most of the antimicrobial films showed more effectiveness against Gram-positive bacteria than Gram-negative bacteria. This is due to the cell wall lipopolysaccharides or the protection of outer membranes of Gram-negative bacteria which could inhibit the diffusion of antimicrobial agent into the cell, thus decreasing the microbial growth inhibition [31].

Based film	Antimicrobial compounds	Loading	Color attributes			Transparency	Refs.
			L*	a*	b*		
Chitosan	Curcumin	0 and 1% (w/w, based on chitosan)	56.24–65.28	(−0.87)–8.17	5.54–47.56	1.36–2.12	[29]
Chitosan	Apple peel polyphenols	0, 0.25, 0.50, 0.75, and 1%	45.52–82.82	(−2.11)–29.17	8.12–47.76	0.71–4.28	[28]
Chitosan	<i>Ziziphora clinopodioides</i> essential oil	0 and 1% (v/w)	84.85–88.66	(−0.12)–(−1.76)	28.15–29.12	—	[34]
Tapioca starch	Grape pomace extracts	0 and 8% (v/v)	73.5–91.5	(−0.32)–10.8	3.91–15.2	0.38–0.60	[32]
Fish myofibrillar protein	Catechin-Kradon extract	0–12 mg/ml	88.92–95.01	(−0.68)–4.84	4.70–18.10	3.35–3.88	[1]
Gelatin	Longan seed extract	50–500 ppm	83.86–89.57	(−0.89)–(−2.20)	11.72–26.43	3.24–3.36	[5]
Gelatin	Grape seed extract	0 and 1% (v/w)	55.12–91.42	(−2.51)–16.77	12.57–15.81	—	[34]

Table 2.
Color attributes and transparency of edible films incorporated with antimicrobial compounds.

Based film	Antimicrobial compounds	Loading	Microorganism(s) tested	Results	Refs.
Chitosan	Curcumin	1% (w/w, based on chitosan)	<i>S. aureus</i> and <i>R. solani</i>	Microorganisms exhibited sensitivity to antimicrobial films	[29]
Chitosan	Propolis extract	2.5–20% (w/w)	<i>S. aureus</i> , <i>S. enteritidis</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Inhibiting all bacteria tested on contact surface	[35]
Chitosan	Turmeric extract	1:2 (v/v, chitosan: extract ratio)	<i>S. aureus</i> and <i>Salmonella</i>	Reduced the count of bacteria tested	[30]
Corn starch-beeswax	Lauric arginate+ natamycin	2000 mg/l + 400 mg/l	<i>R. stolonifer</i> , <i>C. gloeosporioides</i> , <i>B. cinerea</i> , and <i>S. Saintpaul</i>	Completely inhibited all microorganisms tested	[38]
Tapioca starch	Grape pomace extracts	8% (v/v)	<i>S. aureus</i> and <i>L. monocytogenes</i>	Exhibited a stronger inhibitory effect on <i>S. aureus</i> compared to <i>L. monocytogenes</i>	[32]
Chicken feet protein	Marjoram oil	1% (w/v)	<i>E. coli</i> O157:H7, <i>S. enteritidis</i> , <i>L. monocytogenes</i> , and <i>S. aureus</i>	Inhibited all bacteria tested	[14]
Gelatin	Lemongrass oil	5–25% (w/w based on protein)	<i>E. coli</i> , <i>L. monocytogenes</i> , <i>S. aureus</i> , and <i>S. typhimurium</i>	Inhibited all bacteria tested	[2]
Gelatin	Grape seed extract + <i>Ziziphora clinopodioides</i> essential oil	1% (v/w) + 1% (v/w)	<i>S. aureus</i> , <i>B. subtilis</i> , <i>B. cereus</i> , <i>L. monocytogenes</i> , <i>S. typhimurium</i> , and <i>E. coli</i>	Effectively against Gram-positive bacteria	[34]
Fish myofibrillar protein	Catechin-Kradon extract	3–12 mg/ml	<i>S. aureus</i> , <i>E. coli</i> , <i>S. typhimurium</i> , and <i>V. parahaemolyticus</i>	They showed microbial inhibitory effects on the contact surface against all bacteria tested	[1]
Whey protein	Rosemary and thyme extracts	3 and 5%	<i>L. monocytogenes</i> and <i>S. aureus</i>	Inhibited all bacteria tested	[39]

Table 3.
Antimicrobial activity of some compounds used in edible films against some microorganisms.

However, some studies have reported that edible films incorporated with antimicrobial substance did not show any antimicrobial effect on microorganisms. Siripatrawan and Vitchayakitti [35] observed that chitosan films containing propolis extract at different concentrations (2.5–20%, w/w based on chitosan content) did not show any inhibition zone, but they could inhibit bacteria tested on contact surface. They concluded that chitosan polymer and phenolic compounds that present in propolis extract are tightly interacted and led to reduce the release or diffusion of antimicrobial substances from the chitosan film matrix to inhibit bacteria surrounding film disc during agar disc diffusion method.

In addition, the diffusion or releasing of antimicrobial substance through the film is also affected by the composition, manufacturing method, hydrophilic-hydrophobic balance of the antimicrobial agent, and storage conditions [7]. Furthermore, the performance of antimicrobial compounds against the specific or groups of microorganisms and the different interactions among the biopolymer material, the antimicrobial substance, and presented food components also affect this phenomenon [36]. All of these factors can be altered, the antimicrobial activity and the edible films' properties. Therefore, these are being key factors for the making of antimicrobial films.

4. Applications of antimicrobial biopolymer-based film

Appearance, color, and texture are crucial factors that consumers will consider prior to making a decision to buy fresh produce and meat products. Most foods are highly perishable due to their biological and chemical compositions. Pathogen contamination or food deterioration usually occurs on the food surface. When this phenomenon occurred, the food showed undesirable odors and visible changes on the surface of foods. The growth of microorganisms is the most problem of food spoilage which can lower quality and decrease the shelf life and changes in natural microflora that could induce pathogenic problems. Furthermore, microbial growth in foods also significantly reduces the safety of food and the security of public health. Microbial spoilage of foodstuffs is caused by many species of microorganisms, including bacteria, yeast, and molds. However, the food spoilage by microorganisms is dependent upon pH, water activity, nutrients, and presence of oxygen [7].

Various food processing technologies have been developed to prevent the contamination and to inactivate the pathogenic microorganisms. Many nonthermal processing technologies, such as irradiation, high-pressure process, and pulsed electric field, are being studied to estimate their mechanisms and effectiveness in microbial inhibition. However, these technologies may not completely prevent or control pathogenic microorganisms due to the complexity of food composition, a wide variety of microbial physiology, passage of contamination, pathogenic mechanisms, and the mass-production nature of food processing [7]. The use of antimicrobial compounds also reduces or inhibits the microbial population that present in the foods.

The direct inclusion of antimicrobial agents to food products can reduce the antimicrobial activity of antimicrobial compounds because the food components can interfere or reduce their efficiency. Furthermore, some antimicrobial compounds exhibit strong flavor/color which may change the organoleptic properties of food products. Therefore, the antimicrobial film is a promising way to slowly migrate the antimicrobial compound to the surface of foods and enable continued antimicrobial effect on the food surface during extended storage, which may act as additional hurdles against food spoilage. This polymer-based film system can be applied to

various types of food products such as meat, seafood, fruits, and vegetables, in order to maintain quality, enhance food safety, and prolong the shelf life of foods.

In recent years, the applications of antimicrobial films on the real food system were reported. For instance, Putsakum et al. [40] developed 0.3% (w/v) of neem extract (*Azadirachta indica*)-contained gelatin films, applied on minced beef, and determined the quality of minced beef during storage $4 \pm 1^\circ\text{C}$ for 7 days (**Figure 1A**). The results showed that minced beef wrapped with gelatin films containing Neem extract had lower in TBARS value than the sample wrapped with polyvinyl chloride (PVC). Kaewprachu et al. [41] suggested that fish myofibrillar protein films incorporated with 9 mg/ml of catechin-Kradon extract could delay discoloration, lipid oxidation, and the growth of microorganisms throughout the duration of storage (at $4 \pm 1^\circ\text{C}$ for 10 days) (**Figure 1B**).

4.1 Meat and meat-based products

Most of the fresh meat products are highly perishable due to their biological compositions. Fresh meat is generally composed of 12–20% of protein, 0–6% carbohydrates, and 3–45% fat, In fact, muscle tissue is made up of approximately 75.5% water, but this level can range from 42 to 80% [42]. Additionally, the presence of water in meat also affords microorganisms to support their growth. To reduce the growth and spread of pathogenic and spoilage microorganisms in meat foodstuffs, antimicrobial films can be used to inhibit, retard, or kill the growth of microorganisms. Similarly, antimicrobial packaging that releases antimicrobial agents also offers potential for reducing the effect of the growth of slime-forming bacteria on meat surface. Current applications of antimicrobial film on meat and meat-based products are summarized in **Table 4**.

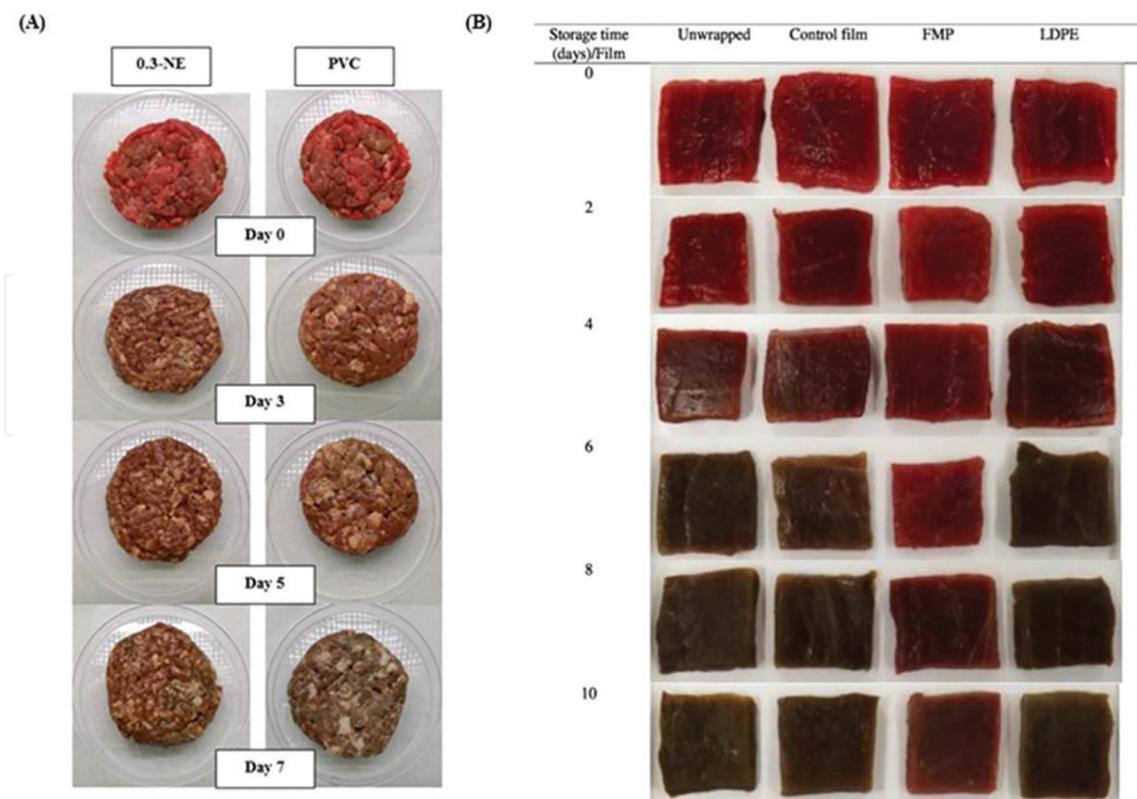


Figure 1. Applications of gelatin films incorporated with 0.3% (w/v) of neem extract (NE) on minced beef during storage at $4 \pm 1^\circ\text{C}$ for 7 days (A) and fish myofibrillar protein (FMP) containing 9 mg/ml of catechin-Kradon extract on bluefin tuna flesh during storage at $4 \pm 1^\circ\text{C}$ for 10 days (B) compared with the commercial wrap films (PVC, polyvinyl chloride and LDPE, low density polyethylene) [40, 41].

Test substrates	Based film	Antimicrobial compounds	Concentration	Results	Refs.
Beef	Whey protein-sodium alginate	Rehydrated supernatant of <i>Lactobacillus sakei</i>	18 mg/ml	Reduced the population of <i>E. coli</i> by 2.3 log CFU/g after 36 h	[43]
Camel meat	Carboxymethyl cellulose	<i>Ziziphora clinopodioides</i> essential oil + <i>Ficus carica</i> extract	2% (v/v) + 1% (w/v)	Reduced the population of spoilage and pathogenic microorganisms	[44]
Chicken meat	Tapioca starch	Grape pomace extracts + cellulose nanocrystal	8% (v/v) + 10% (v/v)	Reduced the growth of <i>L. monocytogenes</i> by 1–2 log CFU/g during 10 days storage	[30]
Chicken fillets	Sodium alginate-galbanum gum	<i>Ziziphora persica</i> essential oils	0.5 and 1.0% (w/v)	Inhibition of natural microflora and <i>Pseudomonas</i> spp. of the chicken fillets	[46]
Ground beef	Cassava starch	Oregano essential oil + pumpkin residue extract	2% (w/v) + 3% (w/v)	Delayed the growth of coliform and <i>Salmonella</i>	[47]
Minced pork	Gelatin	Catechin-lysozyme	0.5% (w/v)	Inhibition of natural microflora of the minced pork	[48]
Pork sausage	Collagen	Nisin	10,000 ppm	Reduced the growth of <i>L. monocytogenes</i> by 1.1 log CFU/g	[49]
Sausage	Calcium alginate	<i>Terminalia arjuna</i> extract	0.5 and 1.0% (w/w)	Inhibition of natural microflora of the sausage	[50]
Ready to cook pork chops	Chitosan	Bamboo vinegar	2% (w/v)	Inhibiting microbial growth against total viable count, lactic acid bacteria, and <i>Pseudomonas</i> spp.	[51]

Table 4.
Application of antimicrobial films on meat and meat-based products.

4.2 Seafood-based products

Seafood products are highly perishable. Different categories of seafood products have unique spoilage patterns based upon innate compositional, chemical biochemical, and microbiological differences [22]. The growth of microorganisms can shorten the shelf life of seafood products by changing the organoleptic properties that affect consumers' acceptability of the products. Also, seafood-associated

foodborne pathogen outbreaks are concern. Most of them are commonly contaminated with several pathogenic microorganisms such as *Vibrio parahaemolyticus*, *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*; however, others such as *Clostridium botulinum* and *Aeromonas hydrophila* are also associated with marine food products [52, 53]. Consumption of the contaminated seafood with pathogenic microorganisms can lead to foodborne illnesses in the form of infection, intoxication, or both [53]. Yücel and Balci [54] evaluated 78 fish samples (30 freshwater and 48 marine fish) for the presence of *Listeria* and *Aeromonas* species from fish market in Ankara, Turkey. The incidence of *Listeria* spp. was 30% in freshwater and 10.4%

Test substrates	Based film	Antimicrobial compounds	Concentration	Results	Refs.
Cold-smoked salmon	Gelatin	Olive leaf extract	5.63% (w/w)	Reduced the growth rate of <i>L. monocytogenes</i>	[52]
Deepwater pink shrimp	Chitosan	Orange essential oil	2% (v/v)	Inhibition of natural microflora of shrimp	[56]
Fish steak	Chitosan	Ginger essential oil	0.3% (v/v)	Inhibition of lactic acid bacteria and <i>B. thermosphacta</i>	[57]
Hake fillets	Whey protein isolate	Oregano and thyme essential oil	1 and 3% (w/w)	Reduction in psychrotrophic bacteria, H ₂ S-producing bacteria, and <i>Pseudomonas</i>	[58]
Shrimp	Gelatin	<i>Ziziphora clinopodioides</i> essential oil + pomegranate peel extract	1% (v/v)	Inhibition of <i>Pseudomonas</i> spp., <i>P. fluorescens</i> , <i>S. putrefaciens</i> , <i>Enterobacteriaceae</i> , and <i>L. monocytogenes</i>	[59]
Shrimp	Chitosan	Pomegranate peel extract	1.5% (w/v)	Inhibition of natural microbiota of shrimp	[60]
Sea bass slices	Fish protein isolate/ gelatin	Basil leaf essential oil	100% (w/w, based on protein)	Retarded microbial growth	[61]
Salmon	Barley bran protein/ gelatin	Grapefruit seed extract	1% (w/v)	Reduced the populations of <i>E. coli</i> O157:H7 and <i>L. monocytogenes</i>	[62]
Tuna slices	Fish myofibrillar protein	Catechin-Kradon extract	9 mg/ml	Inhibiting the growth of microorganisms	[41]
Fatty tuna meat	Red pepper seed meal protein/ gelatin	Oregano oil	0.5% (w/v)	Reduced the growth of <i>L. monocytogenes</i> and <i>S. typhimurium</i>	[63]

Table 5.
 Application of antimicrobial films on seafood-based products.

Test substrates	Based coating	Active compounds	Concentration	Results	Refs.
Apple	Chitosan	Olive oil residue extract	20 g/l	Delayed the growth of <i>P. expansum</i> and <i>R. stolonifer</i> during cold storage (35 days)	[65]
Apple	Pullulan	<i>Satureja hortensis</i> extract	2, 5, 10, and 20% (w/v)	Reduced the growth of <i>S. aureus</i> by 1.4 log CFU/g (at 16°C for 21 days)	[66]
Arbutus berries	Alginate	Eugenol	0.2% (w/v)	Reduced microbial spoilage and arbutus berries can be stored for at least 28 days at 0.5 °C	[67]
Black radish	Chitosan-gelatin	Thyme essential oil	0.2%	Reduction the growth of <i>L. monocytogenes</i> by 2.1 log CFU/g after 24 h	[68]
Lime	Soy protein	Limonene	5 and 10% (w/w, based on protein)	Reduced wounds with <i>Penicillium italicum</i> by 85% after storage at 13°C for 13 days	[69]
Pepper	Pullulan	<i>Satureja hortensis</i> extract	2, 5, 10, and 20% (w/v)	Reduced the growth of <i>A. niger</i> by 1.33 log CFU/g (at 16°C for 21 days)	[65]
Strawberry	Chitosan	Olive oil residue extract	20 g/L	Delayed the growth of <i>P. expansum</i> and <i>R. stolonifer</i> during cold storage (16 days)	[66]
Strawberry	<i>Aloe vera</i>	Ascorbic acid	1, 3, and 5% (w/v)	Reducing microbial populations	[70]

Table 6.
Application of antimicrobial films on fresh and minimally processed fruits and vegetables.

in marine fish samples, while *Aeromonas* spp. isolated from marine fish samples (93.7%) showed higher than freshwater fish (10%), mainly *A. hydrophila*. They also reported that *Aeromonas* spp. can be primary or secondary pathogens of fish. Kahraman et al. [55] reported that the incidence of *A. hydrophila* and *Plesiomonas shigelloides* in 700 seafoods (400 fish, 100 shrimps, and 200 mollusks) was detected in 5.71 and 0.86%, respectively. Thus, the use of antimicrobial film could inhibit the microbial growth, preserve the quality, and prolong the shelf life of seafood. Current applications of antimicrobial packaging on seafood-based products are summarized in **Table 5**.

4.3 Fresh and minimally processed fruits and vegetables

Fruits and vegetables are classified as perishable products. They are very susceptible to biochemical, nutritional, and structural with textural changes. These postharvest changes can be accelerated by loss of water and microorganisms' action. Fungal are mostly associated microorganism outbreak that reduced the quality of fruits and vegetables [64]. The use of antimicrobial films and/or coatings could minimize the undesirable changes in fruit and vegetable; thus, the products have good quality, attractive organoleptic properties, and close to fresh product during

extended storage. Current applications of antimicrobial packaging on fresh and minimally processed fruits and vegetables are summarized in **Table 6**.

5. Conclusions

There are many types of antimicrobial agents that could be incorporated into food packaging materials, especially bio-based films. The suitable selection of antimicrobial substances is crucial to obtain antimicrobial effectiveness. Antimicrobial film is a promising category of active packaging system which able to inhibit the growth of microorganisms and retard the developments of discoloration and off-flavors in food products. The addition of antimicrobial compounds into biopolymer-based edible films could improve the mechanical, water barrier, and antimicrobial properties. Most foods are perishable and are susceptible to microbial contamination. The use of antimicrobial films has shown to preserve quality and increase the shelf life of various food products. The enhancement in the quality of the food products is achieved through inhibiting the target microorganisms.

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