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Chapter

Protein-Based Active Film as Antimicrobial Food Packaging: A Review

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Abstract

This review discusses the protein-based active film as antimicrobial food packaging derived from various sources such as gelatin, casein, whey and zein-based protein. The films properties that exhibit antimicrobial activity are being reviewed along with their application in food packaging industry. This paper also studies the inhibition activity by antimicrobial agents from organic and metallic sources which were incorporated into the protein-based film. Nowadays, protein-based film has emerged as one of the most extensively studied in food packaging sector as it exhibits good mechanical, optical, and oxygen barrier properties. In addition, protein-based film also showed good compatibility to polar surfaces while having effective control on the release of additives and bioactive compounds in food packaging system. This paper also detailed out information on antimicrobial food packaging in order to increase consumer awareness regarding food safety and healthy lifestyle while maintaining the quality and prolonged the shelf life of food product.

Keywords: protein, biopolymer, edible film, active packaging, antimicrobial agents

1. Food packaging

Food packaging is defined as a way of preparing food for transport, distribution, storage, and retailing till the end use while ensuring safe delivery to the ultimate customer [1]. Packaging systems are characterized into three groups which are primary, secondary, and tertiary packaging according to their layers or functions. Primary packaging is the first level of packaging which involves direct contact with the products. While secondary packaging contains a number of primary packages that protect the primary packages from damage during shipment and storage and are also designed to be displayed onto the retail shelves. As tertiary packaging, it acts as distribution carrier which consists of a number of secondary and primary packages [2]. Food packaging is designed to protect and maintain the quality and safety of foods from chemical, biological, and physical deterioration while helping in extending the product's shelf life [3]. In addition, its basic functions are commonly served as containment, protection or preservation, and communication and convenience purpose [1]. Packaging is also helpful in reducing municipal solid waste disposal and the cost of many food products by facilitating large-scale production and efficiency in bulk distribution. Food packaging also ensures the safety of products by decreasing the risk of tampering and adulteration [3].

However, there are major drawbacks regarding non-biodegradable food packaging which caused environmental problems that include changes to the carbon dioxide cycle, composting problems, and increasing level of toxic emissions [4]. Stimulated by the environmental and growing interest of health safety concerns from consumers, many researchers have now been concentrating on ways to develop biodegradable packaging. Food packaging from biodegradable polymers have raised attention due to their renewable and environmental-friendly characteristics. Studies from renewable of natural biopolymers sources were included from polysaccharides (starch and chitin), lipids (waxes and paraffin), proteins (collagen and gelatin), or the combination of these components [5–7]. Among those, protein possessed greater characteristics and potential in food packaging due to its ability in film-forming process with high mechanical and barrier properties [8].

2. Protein-based food packaging

Proteins are composed of amino acid chains linked by peptide bonds to form a primary structure [9]. It can be characterized according to its amino acid composition, geometrical conformation, solubility, molecular weight, sedimentation behavior, surface polarity, and native molecular configuration shape [10]. Protein generally existed in two main classes that are known as fibrous or globular proteins. Fibrous and globular proteins have different sizes, shapes, solubility, appearances, and functions. Fibrous protein served as the main structural materials of animal tissues, while the globular proteins have multiple functions such as formation of enzymes, cellular messengers, and amino acids. Fibrous proteins consist of repetition of a single unit to form chains that act as connective tissues and associated closely with each other in parallel structures to provide strength and joint mobility, while globular proteins consist of long chains with numerous branches and folded into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic, and covalent (disulfide) bonds. The example of fibrous protein that has gained great attention in studies of food packaging material is collagen. As for the globular protein, many research have been conducted on the use of casein, wheat gluten, corn zein, soy protein, whey protein, and mung bean protein as a great potential to be utilized as edible food packaging [11]. In food packaging, protein is mainly used as it exhibits good mechanical, optical, and oxygen barrier properties. Furthermore, protein is able to promote good compatibility to polar surfaces and control the release of additives and bioactive compounds in food packaging system [8, 10]. Therefore, many research have been keen to produce protein-based packaging that emerge in the form of edible films and coatings from various protein sources such as gelatin, casein, whey, corn zein, pea, wheat gluten, amaranth, soy, mung bean, and peanut.

2.1 Protein-based edible film

Nowadays, biodegradable film for food packaging has drawn attention from many researchers as an alternative approach to solve the problem arise from petroleum-based polymeric material that possesses non-biodegradability properties which leads to a critical environmental issue and causes exhaustion of natural resources. Natural biopolymers such as protein are eco-friendly and exhibit nontoxic properties which also have comparable physicochemical characteristics with the synthetic polymeric film [12]. In general, edible film is defined as a standalone thin layer sheet formed from a biopolymer matrix and possessed structural integrity. It served as a moisture barrier or as a solute/gas barrier while being able to improve the mechanical and rheological characteristics of the intended products [13]. These films also help in improving product quality by imparting certain functions such as antioxidant, antimicrobials, or any other specific functions while providing physical protection and extending the shelf life of the food products.

Protein-based films have been extensively utilized due to their relative abundance and good film-forming ability, contain high nutritional value, as well as provide desirable mechanical, gas barrier, and transparency properties [14]. Other than that, protein-based films also showed better mechanical properties than polysaccharide- and lipid-based films due to their unique structure that provides wider range of functional properties especially exhibiting a high intermolecular binding potential that is able to form a bond at a different position [14]. As widely known, protein-based edible film is being utilized from various kinds of protein sources which are mainly classified into two categories: animal and agro-based protein polymers. Several studies that have been conducted on animal-derived protein polymer include collagen, gelatin, fish myofibrillar protein, and whey protein, while the studies on agro-based protein polymer include the sources derived from corn zein, pea, wheat gluten, amaranth, soy, mung bean, and peanut.

The most preferred method to form edible protein-based film is by using solvent casting. The films are formed from solutions or dispersions of the protein as the solvent/carrier evaporates. The solvent/carrier is generally limited to water, ethanol, or ethanol-water mixtures [11]. The method was technically done by spreading dilute film solution and plasticizer into Petri dish or plates and drying them under ambient conditions or controlled relative humidity. Commonly, large-scale production uses more sophisticated equipment that is able to generate larger protein films by mechanically spreading the solution to a fixed thickness. There are several parameters that need to be determined for continuous film production such as air temperature, surface properties of the substrate upon which the films are formed, flow rate, and drying time. The films can be dried under ambient conditions by using several methods that are hot air, infrared energy, or microwave energy. The physical properties of the final film regarding film morphology, appearance, and barrier and mechanical properties can be significantly affected by the drying method used [15]. The other alternative method for protein-based film forming is by using extrusion. Extrusion process used elevated temperature and shear in order to soften and melt the polymer and thus allow the cohesive film matrix to form. The use of extrusion has certain advantages over solvent casting method as it is able to reduce more time and energy inputs as well as raise the cost of biopolymer film formation into a competitive range that is able to match and compete with the synthetic film production [15].

2.2 Protein-based edible coating

Meanwhile, edible coating is a more thinly edible film which is being formed directly onto the food or materials surface [16]. Edible coating can improve the physical and chemical integrity of the product by creating a modified overhead atmosphere and prevent the migration of moisture, oxygen, carbon dioxide, or any other solutes. It also acts as a carrier in terms of food additives (antioxidants, antimicrobials, and specific nutrients) while increasing the shelf life of the product. Furthermore, edible coatings can improve the product's appearance by adding color or gloss which seem to be more appealing to consumers [13, 16].

Protein coating is often being processed by using two common methods that are known as wet and dry (mainly extrusion) processes that depend on the target structure either mono- or multilayer structure. Wet coatings from polymer solutions or suspensions are commonly done by using lacquering or spraying techniques. Rheological properties of the coating formulation are greatly influenced by the techniques that will be used for wet coating process. Different types of methods are applicable for the drying process such as drying under ambient conditions, hot air, infrared energy, or microwave energy. The protein coating properties that include morphology, appearance, and barrier and mechanical properties will be influenced by the drying method used. As for dry process, extrusion method is one of the most common techniques that is being applied in conventional industrial method for protein coating. An extruder works by allowing the polymer to melt at high temperature during a relatively short time. The mechanical action of the screw and temperature exerts the material to melt, convey, compress, shear, mix, undergo variation of its amorphous content, optionally react, and be finally shaped through a die of a desired shape [10].

The protein-based film and coating were commonly being tested on their mechanical (tensile strength, elongation at break, and Young's modulus), barrier, (water vapor permeability and oxygen permeability), and physical (color and transparency) properties. However, due to its hydrophilic nature, protein-based film and coating also have high sensitivity to moisture and poor water vapor barrier properties. Thus, many studies have been conducted in order to improve and modify the functionality of protein-based film and coating as food packaging which includes the addition of different substances or agents such as cross linkers, plasticizers, and additives with antioxidant and antimicrobial properties. The incorporation of certain additives into packaging systems that intended to maintain or extend the quality of product or shelf life is referred as active packaging [17].

3. Active packaging

Active packaging is a medium which allows the interaction between the packaging, product, and environment. These systems involved the chemical, physical, and biological activities which change conditions of the packed food and help in extending the product's sustainability and shelf life. Moreover, active packaging is also able to enhance the microbiological safety and the sensory properties while maintaining the quality of the intended product [18]. Commonly, active packaging systems are concerned with substances that absorb (scavengers) or release (emitters) gases or steam which actively modifies the atmosphere inside packaging. Scavengers are used to remove unwanted items that commonly involved with the absorption of oxygen, ethylene, moisture, carbon dioxide, and flavors/odors from the environment into the internal packaging, while emitters are designed to release desired items that have a positive impact on food into the packaging environment that are commonly associated with the emitter of carbon dioxide, antimicrobial agents, antioxidants, and flavors [18]. Among those, antimicrobial packaging has been considered as the most promising method which incorporated antimicrobial agents into food packaging system that help in controlling the undesirable growth of a microorganism while extending the product's safety and shelf life [19]. As protein structure is comprised of hydrophilic nature, it can allow the control release of additive and bioactive compounds which make the protein-based film as one of the most promising media to be used in designated active antimicrobial packaging application.

3.1 Protein-based film as active packaging

Protein-based edible films were usually made from protein solutions or dispersions as the solvent/carrier evaporates. The solvent/carrier is normally composed of either water, ethanol, or ethanol-water mixtures [11]. Even though they exhibit poor water resistance, however, they are better when compared to polysaccharides in film-forming ability with good mechanical and barrier properties [20]. Protein-based film as active antimicrobial packaging is designed based on the diffusion of incorporated antimicrobial compounds to the product's surface while aiming to extend the shelf life period [21]. The antimicrobial activity depends on the rate of active compound diffusion by the antimicrobial agents which depends on several factors such as chemical compatibility with polymer matrix, headspace humidity, the physicochemical properties of the product which is being tested on, antimicrobial solubility in tested food, and also the released temperature [21]. Active antimicrobial packaging from protein-based edible films can be derived from various sources such as gelatin, casein, whey, corn zein, and wheat gluten.

3.1.1 Gelatin-based film as active packaging

Gelatin is a protein obtained by hydrolyzing the collagen contained in bones and skin of animals. Physical and chemical properties of the gelatin produced are greatly affected by the sources, age of animal, collagen type, and extraction method used [22]. The global gelatin production was 348.9 kilo tons in 2011 and is expected to reach 450.7 kilo tons in 2018, growing at a compound annual growth rate (CAGR) of 3.73% from 2012 to 2018 [23]. Among all protein sources, gelatin is being one of the most extensively studied due to its good filming properties while performing its duties to protect and extend the shelf life of food products. Many antimicrobial agents have been incorporated into a gelatin-based film such as metal ions, essential oils, natural extracts, polymers, organic acids, and bacteriocins which resulted in great inhibition toward growth of microorganism and pathogens.

For example, the gelatin-based active nanocomposite films containing silver nanoparticles (AgNPs) resulted in high antimicrobial activity against both Gram-negative (*Escherichia coli*) and Gram-positive (*Listeria monocytogenes*) bacteria. The bacterial inhibition might be due to the interaction of AgNPs with phosphorous and sulfur binding to microbial DNA and prevents bacterial replication which leads to cell death [24]. While, gelatin-based film with incorporation of oregano essential oil is found to be effective against Gram-negative (*Salmonella enteritidis* and *Escherichia coli*) and Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) bacteria. These antimicrobial properties might be attributed by the presence of two phenols (carvacrol and thymol) and monoterpene hydrocarbons (p-cymene and γ-terpinene) compounds which are present in oregano essential oils [25]. Based on another study, the incorporation of citric acid into the gelatin-based film also showed reduction of growth for Gram-negative bacteria (*Escherichia coli*) [26]. Citric acid has pKa 4.8 that makes cell membrane become permeable and allows the acid to enter the cell. Upon entering the cytoplasm, the acid will dissociate, thus lowering the internal pH of the cell which leads to disruption of cellular functions of a microorganism [27]. Based on the results of this study, it showed that gelatin with the incorporation of antimicrobial agents has resulted in excellent properties as active antimicrobial food packaging as they managed to inhibit microbial growth of the food product. Thus, it can be concluded that gelatin-based film has emerged as one of the most widely studied biopolymer in film processing sector as compared to other sources of protein-based film while showing great potential as a medium to release or emit active antimicrobial agents against growth of microorganism and pathogens.

3.1.2 Casein-based film as active packaging

Casein-based edible film production also has been numerously studied because they displayed high nutritional quality with good sensory properties. Casein is commonly found in mammalian milk or in dairy products. Casein proteins comprise 80% of the total protein content in milk which precipitated from skim milk by acidifying the milk to produce acid casein to its isoelectric point of approximately 4.6 or the milk is treated with rennet to produce rennet casein. The casein is then being separated, washed, and dried [11, 28]. Casein is mainly comprised of three principal components, α, β, and κ, that formed colloidal micelles in milk which contains numerous amounts of casein molecules that are being stabilized by a calcium-phosphate bridge [11]. Due to excellent functional properties and natural abundant sources, caseins are used in numerous manufactured products such as in bakery applications, beverages, milk product, snack foods, edible films, etc. Casein or caseinates in the world market used in the food industry were reported in the range between 200,000 and 2,500,000 tons [28]. Caseins and caseinates can be prompted into edible films from aqueous solutions. Edible casein films are able to form a good barrier against oxygen and other nonpolar molecules because casein helps in supplying a great quantity of polar functional groups, such as hydroxyl and amino groups toward the film matrix. This property allows the casein film to be used as active packaging and can be combined with other packaging materials to protect products which are prone to oxidation or moisture [29].

In a study conducted by Arrieta et al. [30], sodium and calcium caseinate films with addition of carvacrol showed antibacterial effectiveness against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria, while sodium caseinate-based edible film containing *Zataria multiflora Boiss*. essential oil exhibited a large inhibitory effect on Gram-positive (*Staphylococcus aureus*) followed by Gram-negative (*Salmonella Typhimurium* and *Escherichia coli*) bacteria [31]. Meanwhile, Oussalah et al. [32] mentioned that calcium caseinate and whey protein isolate edible films containing carboxymethyl cellulose with addition of 1% oregano essential oil showed inhibitory effect against Gram-negative bacteria that were *Escherichia coli* and *Pseudomonas* spp. on the surface of beefsteaks. The antimicrobial activity was mainly derived from phenolic compounds (carvacrol and thymol) which are present in the essential oil. The inhibition effect was done by interacting with the lipid bilayer of cytoplasmic membranes, causing them to be more permeable, which later induced and increased uptake of antibiotics by the bacterial cell [33]. From this study, it can be seen that caseinate film provides good matrices for the antimicrobial agent to release the active compounds that help to inhibit the growth of microorganisms.

3.1.3 Whey protein-based film as active packaging

Whey is a by-product derived from cheese-making process which is being defined as the remaining matter in the milk serum after coagulation of casein at pH 4.6 and temperature of 20°C. Whey protein is comprised of several individual proteins known as beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, and immunoglobulins [15]. The global whey protein market is projected to reach the compound annual growth rate (CAGR) of 7.5% from 2018 to 2023, and the demand was estimated at a value of $9.4 billion in 2017 [34]. The recovering process of whey solid component helps in reducing the organic pollution evolved from whey wastes while being able to optimally utilize the nutritional and functional properties provided by whey protein to be used in diverse sector [35]. The demand for whey protein among producers of food and beverages is increasing as they capitalize on the functional benefits of whey protein in various products such as sports nutrition, confectionery, bakery and ice cream products, infant formula, and health foods. Recent study has developed an alternative use of whey protein products to form edible film and coatings on surface of food products [36]. Whey protein-based films are found to exhibit clear, odorless with good barrier properties to oxygen and lipids [37]. They also provide good matrices which allow the combination with other packaging materials to enhance the film's functionality as an active film against microorganism or moisture.

A study reported that whey protein-based films incorporated with oregano and garlic essential oil resulted in larger inhibitory zones on Gram-negative (*Escherichia coli* and *Salmonella Enteritidis*) and Gram-positive bacteria (*Staphylococcus aureus*, *Lactobacillus plantarum*, and *Listeria monocytogenes*) [38], while another study of whey protein-based films incorporated with oregano essential oil showed antimicrobial activity against fungus species (*Penicillium commune*) [39]. The inhibition toward the microorganism was prompted by thymol and carvacrol compounds that are mainly present in essential oil. Based on another study of whey protein isolate (WPI) films supplemented with *Lactobacillus sakei*, the bacterial reductions were observed for Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Listeria monocytogenes*) after 36 hours and 120 hours of refrigerated storage on beef cube sample, respectively [40]. *Lactobacillus sakei* produced bacteriocin known as sakacin P which tends to exhibit antimicrobial properties [41]. Meanwhile, the incorporation of acetic, lactic, propionic, and benzoic acids (5%, v/v each) into whey protein-based edible film showed great inhibition zones against Gram-negative (*Escherichia coli* and *Salmonella* sp.) and Gram-positive bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) [42]. The use of acids causes acidification of growth media through acid dissociation into the cytoplasm which then induced the microbial inhibition [27]. Whey protein-based film also yields excellent results toward growth of microorganism as it provides good polymeric matrices for the antimicrobial agents to emit the active compounds into the packaging system.

3.1.4 Zein protein-based film as active packaging

Zein is a major protein in corn that is being classified as prolamin protein which dissolved in 70–80% ethanol. Zein is a relatively hydrophobic and thermoplastic material. The high content of nonpolar amino acids found in zein component might have an association with the hydrophobic nature of zein [11]. The corn is processed by using four different methods: wet-milling, dry milling, dry-grind processing, and alkaline treatment. After that, zein is being extracted from these products/coproducts of corn which could result in different properties and end uses. Corn wet-milling process yields a protein-rich coproduct called corn gluten meal (CGM) from which zein has been commercially extracted. The other methods are dry-milled corn (DMC) in which fibrous material is being separated from grits. As for the dry-grind ethanol process, the corn is ground along with the subsequent saccharification and fermentation of glucose to ethanol, leaving behind the coproduct distillers' dried grains with solubles (DDGS). Fractions such as cellulosic materials and protein are concentrated in DDGS due to conversion of starch to sugars and subsequently ethanol. While, alkaline treatment method has been mainly utilized for the use of human consumption and only has little basis for zein extraction. Most zein extractions have been based on aqueous alcohol extractions, but many other solvents were reported to be able to solubilize zein too [43]. A report by Informa Economics, Inc. [44] showed that zein was clarified as high-value product, and the cost for purified zein production had achieved \$9–30/lb. Zein proteins have been found to serve good materials for coating in pharmaceutical products and food ingredients as they exhibit tough and hydrophobic grease-proof coating properties that make them resistant against microbial attacks. Other potential applications of zein include its usage in fiber, adhesive, coating, ceramic, ink, cosmetic, textile, chewing gum, and biodegradable plastics [45]. In addition, numerous studies have been conducted on utilization of zein protein on development of biodegradable films as it exhibits good film-forming properties. Zein film production involves the development of hydrophobic, hydrogen, and limited disulfide bonds between zein chains in the film matrix [11]. Moreover, zein also showed good properties as carrier for antimicrobial agents such as lysozyme, lactoperoxidase, glucose oxidase, bacteriocins, plant phenolics, and essential oils [20].

A study conducted by Moradi et al. [46] has proved that zein-based film showed excellent antimicrobial properties through the release time of antibacterial agent from the film matrix into minced meat. In this literature, the zein and *Zataria multiflora Boiss.* essential oil-incorporated film has resulted in effective inhibition against both Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Listeria monocytogenes*) during 3 days of storage at 4°C. The result obtained corresponded with the finding by Kashiri et al. [21] which stated that the results showed that films containing *Zataria multiflora Boiss*. essential oil at 5% (g of essential oil/g of dry zein powder) achieved reductions of 1.18 log and 1.14 log against Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli*) bacteria, respectively. While, as the concentration *Zataria multiflora Boiss*. essential oil being increased to 10%, the log reduction value increased to 2.16 log and 2.65 log for films against Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli*) bacteria, respectively. From this study, the antimicrobial effect can be explained by the major compound (thymol and carvacrol) found in *Zataria multiflora Boiss.* essential oil which prevents the further growth of microorganism. The study also focused on addition of monolaurin into zein-based film which resulted in effective antimicrobial activity against Gram-positive bacteria (*Listeria monocytogenes*) during 3 days of storage at 4°C. The inhibition effect toward microorganism by monolaurin is caused by the interference with cytoplasmic membrane of microorganisms [46]. In another study by Mei et al. [47], the antimicrobial activity of silver nanocluster (AgNCs) and $AgNO_3$- embedded zein film was tested on pathogenic *Escherichia coli*. The study showed that there were inhibition zones present with 1.95 and 2.05 mm at concentration of 10 μg Ag of AgNCs and $AgNO_3$, respectively. Silver particles exhibit great antimicrobial activity as they can bind to the bacterial cell wall and cell membrane and inhibit the respiration process of microorganisms, while for the case of *Escherichia coli* inhibition, silver inhibits the uptake of phosphate while releasing succinate, proline, phosphate, mannitol, and glutamine from *Escherichia coli* cells [48]. Thus, based on all the results obtained by this study, it can be concluded that zein-based film does result in good compatibility with incorporation of antimicrobial agents. This is because the antimicrobial agents are able to release the antimicrobial compound agent into the packaging system and managed to inhibit the growth of microorganism.

4. Antimicrobial agent in food packaging

Active antimicrobial packaging involved the continuous interaction with the food product over specific shelf life by actively altering the internal environment [49]. In antimicrobial packaging system, the prevention and reducing growth rate of microorganism by extending the lag period will occur once the antimicrobial agents have been acquired [50]. An extensive study discussed the incorporation of antimicrobial agents in food packaging system from various organic and inorganic sources such as natural extracts (green tea), essential oils (clove, oregano, and thyme), enzyme (lysozyme), polymer (chitosan), organic acid (acetic acid, lactic acid, and benzoic acid), bacteriocins (nisin), and metal ions (zinc oxide and silver nanoparticles). However, due to stability of organic sources when exposed to extreme temperature, their use and application as antimicrobial agents might be limited. Thus, inorganic metals have gained more interest as they are relatively more stable at higher temperatures [51].

4.1 Organic sources as antimicrobial agents

Organic sources of antimicrobial agents can be comprised of animal and plant origin, microbial metabolites, and organic acids. As majority of organic antimicrobials derived from natural origin, they are thus able to inactivate microorganisms and enzymes without affecting the organoleptic or nutritional properties of the food products.

4.1.1 Plant-derived antimicrobial agent

Plant-derived antimicrobial agents possessed phenolic compounds that are able to alter the permeability of microbial cell and interfere with cell membrane functionality such as electron transport, protein synthesis, nutrient uptake, and enzyme activity, while the phenolic compounds also allow the loss of biomolecules such as ribose and sodium glutamate from inside the cell [52]. A wide range of study on antimicrobial agents that derived from plant origin mainly discussed on essential oils and natural extracts. The antimicrobial activity of essential oils is based on their molecular hydrophobicity which allows strong interaction with the lipids of cell membrane through their existence of phenolic compounds. This action will then increase the permeability of cell membrane and disturb the functionality and structure of the cell which leads to leakage of ions and cytoplasmic content inside the cell [53].

In a study conducted by Yanwong and Threepopnatkul [54], the fish skin gelatin-edible films were incorporated with peppermint and citronella essential oils at different concentrations (10, 20, and 30%, w/w). The study showed that the incorporation of both essential oils exhibited excellent antibacterial properties against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. The inhibition activities were triggered by the presence of a major constituent in both essential oils that were identified as *p*-menthone and menthol and citronellal and citronellol for peppermint and citronella essential oils, respectively [55, 56]. While, Martucci et al. [57] observed the antimicrobial activity of oregano and lavender essential oils incorporated into gelatin film that were tested against *Escherichia coli* and *Staphylococcus aureus*. The study found that both essential oils exhibited good antimicrobial properties against the tested bacteria which were Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) in concentrations above 2000 ppm. Oregano essential oil contained carvacrol and thymol, while lavender essential oil revealed a prevalence of linalool and camphor as their major compounds which exhibited good antimicrobial activity against tested microorganism. Another study mentioned that whey protein film with addition of 1–4% of cinnamon oil was tested against *Escherichia coli* and *Staphylococcus aureus*. However, the study showed antimicrobial activity only against *Staphylococcus aureus* with the highest inhibition zone at 4% addition of cinnamon oil into the whey protein film [58]. In addition, a major compound in cinnamon oil that exhibited the antimicrobial activity was identified as trans-cinnamaldehyde or cinnamaldehyde [59]. In the literature, it was mentioned that *Escherichia coli* exerted more resistance to cinnamon essential oil as compared to *Staphylococcus aureus*. This is due to the difference in bacteria's outer membrane structure. Gram-negative bacteria (*Escherichia coli*) possessed a thicker layer of the lipopolysaccharide outer membrane around the cell wall which is shown to be more resistant to hydrophobic substance of essential oil as compared with the Gram-positive (*Staphylococcus aureus*), which possesses single peptidoglycan layer structure [59]. Thus, from this study, it can be seen that all mentioned essential oils exhibited certain compounds that exerted good antimicrobial activity which could enhance the food product shelf life and stability.

4.1.2 Animal-derived antimicrobial agent

Antimicrobial agents originated from animal sources are commonly being used as they exhibited good resistance and inhibition toward growth of microorganism. They evolved as part of defense mechanisms in antimicrobial system. Most of the antimicrobial agents derived from animals emerged in the form of antimicrobial peptides such as pleurocidin, lactoferrin, defensins, and protamine [52]. These peptides were applicable as antibiotic resistant as they are able to destruct the cellular lipid bilayer membranes and can hinder even the fast-growing microorganism to mutate. Furthermore, they have good antimicrobial activity against both Gram-positive and Gram-negative bacteria while also showing antifungal and antiviral activities [60]. There are other effective antimicrobial enzymes which come from egg white, milk, and blood that are known as lysozyme. A study by Kaewprachu et al. [61] reported that minced pork wrapped with catechin-lysozyme which incorporated with gelatin film resulted in lower counts of total plate count, yeasts, and molds than minced pork that was wrapped with PVC film. The addition of lysozyme triggered the cleaving process of peptidoglycan in bacterial cell walls and resulted in lysis of bacterial cell. This result showed that catechin-lysozyme/gelatin film could inhibit the microbial growth, while there are also various studies on certain polysaccharides and lipids from animals that showed excellent antimicrobial activity. For example, Pisoschi et al. [52] stated that chitin derived from the exoskeletons of crustaceans, insects, mollusks, and the cell wall of microorganisms exhibited antimicrobial properties. Apart from that, chitosan which is obtained from the exoskeletons of crustaceans and arthropods and existed as a deacetylated form of chitin also showed effective antifungal and antibacterial activities [52]. In a literature studied by Malinowska-Pañczyk et al. [62], the incorporation of chitosan was observed on its ability as antimicrobial agent against Gram-negative (*Escherichia coli* and *Pseudomonas fluorescens*) and Gram-positive (*Staphylococcus aureus* and *Listeria innocua*) bacteria. The study then revealed all strains of bacteria were completely inactivated after 24 hours of incubation period for gelatin film incorporated with chitosan-90. As for gelatin films with addition of chitosan-73, the results showed that only *Pseudomonas fluorescens* and *Listeria innocua* were completely inactivated, while *Escherichia coli* and *Staphylococcus aureus* cells were partially inactivated after 24 hours of incubation. The inhibition factor was due to the cationic nature of chitosan which induced the electrostatic interaction between positively charged RN $(CH_3)_3^+$ sites and negatively charged microbial cell membranes which led to cellular lysis [63]. Therefore, it can be seen that antimicrobial agents derived from animals resulted good inhibitory effect against microorganism and can be applied in food packaging for enhancing shelf life and quality of intended products.

4.2 Metallic sources as antimicrobial agents

Metals have been widely used as antimicrobial agents for a long time due to their ability to cause injuries to microbial cells by exerting oxidative stress, protein dysfunction, or membrane damage [64]. Metal ions such as copper, silver, zinc, palladium, and titanium have been studied as active antimicrobial agents against a wide spectrum of bacteria, yeast, and fungi [65]. Due to stability of organic sources at higher temperature, their application in food packaging may be limited, thus giving great advantage to metallic sources that are more stable at higher temperature [51].

4.2.1 Zinc particles as antimicrobial agents

Zinc particle especially zinc oxide (ZnO) is being widely proposed to be used as antimicrobial agents with broad range of other applications due to their specialty to survive under harsh environment. The antimicrobial activity by zinc oxide (ZnO) particles were proposed due to emission of zinc ions (Zn^{2+}), which are able to penetrate into the bacteria's cell wall and affect the cytoplasmic content in the cell that leads to the death of bacteria. The incorporation of zinc oxide nanoparticles into gelatin was observed by Divya et al. [66] which revealed that the film showed higher inhibitory effect against Gram-negative bacteria (*Pseudomonas aeruginosa*) than Gram-positive (*Enterococcus faecalis*) bacteria. The results corresponded with the statement which suggested that ZnO induced photocatalytic mechanism related to the semiconductive properties of ZnO which lead to the formation of reactive oxygen species (ROS) and H_2O_2 which damaged the cell wall structure of bacteria [67, 68]. The literature by Pasquet et al. [67] also stated that the lipid bilayer membrane of Gram-negative bacteria was more sensitive toward reactive oxygen species (ROS) produced by ZnO particles than the thick membrane of Gram-positive bacteria that is coated by a peptidoglycan protective layer. Meanwhile, the study on gelatin/ZnO nanoparticles of nanocomposite films showed great antibacterial activity against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli*) foodborne pathogenic bacteria. The study discussed that the antimicrobial activity toward both Gram-negative and Gram-positive bacteria was due to the easy penetration of nanoparticles in the cytoplasmic content of the cell which then leads to death of cell [69]. Thus, it can be concluded that incorporation of zinc particles into protein-based film helps in exerting antimicrobial activity against foodborne pathogens.

4.2.2 Silver particles as antimicrobial agents

The application of silver particles has received great attention from the researchers from all over the world due to their wide spectrum and application in antimicrobial packaging. Silver is often used in the size of nanoparticles as they give more potent effect against foodborne pathogen due to their enhanced catalytic reactivity owing to its large surface area to volume ratio. Based on a study conducted by Kanmani and Rhim [24], the incorporation of silver nanoparticles (AgNPs) into gelatin film was tested against Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli*) bacteria. The results mentioned that the AgNP/gelatin biocomposite film showed high-inhibitory effect against both tested microorganisms. This might be due the interaction of AgNPs with compounds of protein and DNA in the cell that contain phosphorous and sulfur which prevent DNA replication and cause death of the cell. Furthermore, some study suggested that positively charged AgNPs are able to bind with negatively charged bacterial cell membranes that cause disruption of cell walls by shrinkage of the cytoplasm and membrane detachment that led to cell death [70]. In this literature, it also stated that AgNPs could penetrate the bacteria which inactivate the enzymes and induce the production of H_2O_2 and cause cell to die [24]. Meanwhile, another study by Mei et al. [47] observed on the antimicrobial activity of silver nanocluster (AgNC)-embedded zein film against pathogenic *Escherichia coli*. The study showed that adding 10 μg of AgNCs into zein film resulted in great antimicrobial effect as indicated by inhibition zones of 1.95. In this literature, it stated that the inhibitory effect was influenced by the release rate of Ag that was embedded in zein films. Since AgNCs had high surface to volume ratio due to its ultra-small size, it thus led to greater surface contact with bacteria and consequently exhibited higher antimicrobial activity. Therefore, based on this study, it can be confirmed that addition of silver particles into protein-based film is able to exhibit mechanism of antibacterial action against microorganisms.

5. Application of protein-based active film in food packaging

Protein-based edible film has gained great interest due to its wide application as edible food packaging as compared to synthetic films. In addition, it is able to provide good matrix and acts as a medium for incorporation of antimicrobial and antioxidant agents into the film to release or emit their specific functions that help in enhancing the safety, stability, functionality, and shelf life of food products. Moreover, it also can be applied to control the diffusion rate of preservative substances from the product's surface to the internal environment of food. Meanwhile, protein-based packaging also is being extensively used as food wrapper and is being applied at the interfaces between different layers of heterogeneous food, while protein-based edible films could be used together with nonedible film as multilayer food packaging materials where it can be employed as internal layers that have direct contact with food materials [11]. Furthermore, due to good permeability against oxygen, carbon dioxide, and water vapor, protein-based film can be applied to the surfaces of fresh-cut food product in order to extend shelf life of the product by delaying color changes and ripening and prevent the effect of enzymatic browning and reducing moisture and aroma loss [71]. Thus, it can be concluded that protein-based edible film exhibits good characteristics through their mechanical and barrier properties as food packaging which is able to substitute the utilization synthetic film packaging.

6. Conclusion

Antimicrobial food packaging have gained great interest due to high inhibition of microbial activity that helps in prolonging the shelf life of packaged food and enhancing the food's safety while improving the functionality of the film. The incorporation of antimicrobial agents from various organic and inorganic sources into protein-based edible film has been discussed, and their effectiveness was mainly found depending on their activity against the target microorganism, the types of polymer used, the film's properties, and the factor based on the packaged food's composition, pH, water activity, as well as the storage and environmental condition. However, there are some challenges in creating good antimicrobial films that follow the regulatory and industry requirements which also aim in producing at low production cost and are able to meet with the consumer demands without altering the sensory characteristics of the intended packaged food. Therefore, more studies need to be done on biocomposite protein-based packaging films with incorporation of antimicrobial agents that might also require chemical, toxicological, and further test in securing more safe and approved products according to the standard food safety regulations while being able to deliver good means in protecting the safety and quality of packaged food.

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