Chapter 1 Bio-based antimicrobial packaging: A response to a reduction in the use of plastics and an advance in food safety. A review

Capítulo 1 Empaques antimicrobianos de base biológica: Una respuesta a la reducción del uso de plásticos y un avance en la inocuidad de los alimentos

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Abstract

Packaging has been developed to facilitate the transport, handling of food and providing a barrier against external factors. However, it has led to an increase in municipal solid waste (MSW) caused by plastics and waste produced during transport and distribution, which has prompted the development of bio-based antimicrobial packaging (BBA). One of the functions of BBAs is to inhibit the growth of microorganisms and reduce environmental contamination by using biodegradable materials, which is why the development of this type of packaging has become of great interest for research. This compilation provides an overview of the importance of BBAs, the methods and materials used for their production. Also, the most studied antimicrobial agents, their effect on the mechanical and barrier properties of packaging, and the advances have been made in BBAs.

Antimicrobial packaging, Biopolymers, Antimicrobial agents

Resumen

Los empaques han sido elaborados para facilitar el transporte y el manejo de los alimentos, además de aportar una barrera contra los factores externos, sin embargo, ha producido el aumento en los residuos sólidos urbanos (RSU) causados por los plásticos y los desperdicios producidos durante su transporte y distribución, lo que ha impulsado al desarrollo de empaques antimicrobianos de base biológica (EAB). Una de las funciones de los EAB es reducir o inhibir el crecimiento de microorganismos, además de disminuir la contaminación ambiental al utilizar materiales biodegradables, por ello en la actualidad el desarrollo de este tipo de empaques ha cobrado gran interés para la investigación. El objetivo de esta recopilación es proporcionar un panorama general de la importancia de los EAB, los métodos y materiales utilizados para su producción, los agentes antimicrobianos más estudiados y su efecto en las propiedades mecánicas y de barrera en los empaques, así como algunos de los avances que se han tenido en el área de EAB.

Empaques antimicrobianos, Biopolimeros, Agentes antimicrobianos

1 Introduction

For several decades, packaging has facilitated the transport and preservation of food. They have to fulfill specific functions such as containment, protection, convenience and communication. This last function refers to how the consumer identifies the product from others and provides information such as the nutritional table and traceability (Coles & Kirwan, 2011; Robertson, 2013; Singh *et al*., 2017). According to FAO, global food losses and waste are estimated at 1.3 million tons per year, implying a high carbon footprint. The industry's demand to reduce these losses led to changes in the manufacture of packaging, which promoted the development of active packaging (AP), which, in addition to fulfilling basic functions, are designed specifically for the needs of the product. Their importance lies in incorporating active substances in the packaging material, thus stabilizing changes that reduce food quality. The AP can be classified into two major systems: those that absorb oxygen, ethylene, moisture, carbon dioxide, flavors/odors; and those that release carbon dioxide, antimicrobial agents, antioxidants and flavors (Vermeiren, Devlieghere, Van Beest, De Kruijf & Debevere, 1999; FAO, 2011; Guillard, Gaucel, Fornaciari, Angellier-Coussy, Buche, & Gontard, 2018).

Active packaging has been studied to retard food spoilage. Such is the case of Hutter and Yildirim (2016), who evaluated the discoloration of ham by using palladium in active films. Their results show that when storing the food in palladium-containing films, the Δa^* values increased. This is due to the absence of oxygen by palladium which prevents discoloration caused by the oxidation of nitrosomyoglobin. Another study is Bovi *et al*. (2018), who prepared active films using fructose as a moisture absorber. Their results showed that adsorbed moisture was higher when using 30 % fructose at 20 °C. In addition, they found that fructose packaging minimized condensation inside the packaging, favoring the preservation of strawberries during storage. Jiang *et al*. (2020) studied the effect of adding lemon essential oil to grass carp collagen films. They observed more efficient preservation of pork meat with the oil-added films. Peroxide values were lower in the active films (7.13±0.85 meq kg^{-1}) than the grass carp collagen films (11.35±1.04 meq kg^{-1}), indicating less lipid peroxidation in the frozen meat during storage. Further studies conducted in recent years on AEs, their function, and their food applications are presented in Table 1.1.

Table 1.1 Examples of active packaging and some food applications

Function of packaging	Active agent	Application in food	Author
Oxygen scavenger	Iron powder	Sausages	Gibis & Rieblinger, 2011
Oxygen scavenger	Palladium	Slices of ham	Hutter & Yildirim, 2016
KuEthylene scavenger	Clay nanoparticles	Banana, Strawberry and tomatoes	Tas *et al*., 2017
Ethylene scavenger	TiO_2	tomatoes	Kaewklin *et al*., 2018
Moisture absorber	Cellulose/fructose	Strawberry	Bovi *et al*., 2018
Moisture-absorbing and antioxidant	Green tea/PVA	Dried eel	Chen *et al*., 2017
Antioxidant	Rosemary extract polyphenols	Fat food simulator	Piñero-Hernández *et al*., 2017
Antioxidant	Lemon essential oil	Pork	Jiang *et al*., 2020
Antioxidant and antimicrobial	Clove essential oil	Sardine pancakes	Salgado *et al*., 2013
Antimicrobial	Potassium sorbate or vanillin	Butter cake	Sangsuwan, Rattanapanone & Pongsirikul, 2014
Antimicrobial	Acrylonitrile and acrylamide	Apple and guava	Kumar, Kumar & Pandey, 2018
Antimicrobial	Eugenol	Lateolabrax japonicus	Li *et al*., 2019

Antimicrobial packaging is characterized by inhibiting the growth of microorganisms that can reduce food quality or cause disease by incorporating enzymes, bacteriocins or essential oils that control spoilage microorganisms and pathogens that cause foodborne illnesses (ETAS) (Alvarez, 2000; Cha & Chinnan, 2004).

According to WHO reports, in 2018, 550 million people fell ill due to contaminated food. Furthermore, figures reported in the World Bank show a productivity loss due to ETAS of $92.2 billion per year. In 2019, at the international conference on food safety, they reiterated the importance of safety and implementation of measures in food to reduce the incidence of ETAS. For this reason, antimicrobial packaging is of great importance.

In addition to ETAS and the losses caused by them, there are also contamination problems caused by plastics, which is the main component currently used for the manufacture of packaging in food. In 2015 and 2016 alone, a worldwide increase of 4.2 % in plastic production was reported, equivalent to 335 million tons. In 2018, Sardon and Dove revealed that by 2050 its production would exceed 500 million metric tons, alarming figures due to its persistence and effects on the oceans, wildlife and humans. The increase in the production of plastics, combined with their short lifespan and poor recycling mechanisms, will lead to a scenario where, by 2050, there will be more plastic than fish in the sea, which is why several studies are being conducted for the use of different polymers from biological sources to replace plastics (Jambeck *et al*., 2015; Guillard *et al*., 2018; Sardon & Dove, 2018).

The BBA has been developed to solve this problem. They are formulated with antimicrobial agents that inhibit the growth of microorganisms that cause ETAS and are added to polymeric matrices of biological nature that are degraded to natural compounds such as CO_2, CH_4, and organic compounds. Hence, they turn out to be an attractive alternative due to their ecological, renewable, economic and biocompatible characteristics (Zhong, Godwin, Jin, & Xiao, 2019). For this reason, this chapter aims to give an overview of the impact of BBA, the methods for their production, the main bio-based matrices and antimicrobial agents used in their formulation and their effect on the mechanical properties of the packaging, as well as future advances in antimicrobial packaging technology.

2. Bio-based antimicrobial packaging

The growth of microorganisms causes spoilage in food products. Traditional preservation methods such as drying, heating, freezing, fermentation and salting extend the shelf life of foods, but they must be packaged to avoid contamination and facilitate handling. For this reason, different preservation methods are currently being combined to extend the shelf life of products without changing their composition. Within these technologies, we can define *antimicrobial packaging* as a packaging system that interacts with the product or headspace to inhibit microorganisms present in the product (Han, 2005; Yam & Lee, 2012).

Antimicrobial packaging extends the dormancy period and reduces the growth rate of microorganisms to prolong their shelf life and maintain food safety. The efficiency of antimicrobial packaging is determined by the release of the antimicrobial, which is slow and gradual to maintain its effectiveness over a prolonged time. Antimicrobial packaging only inhibits the growth of microorganisms but must also meet several vital requirements; use materials that are authorized to be in contact with food, must be simple and cost-effective, chemically stable for prolonged use and act as a barrier to gases and water vapor.

3. Methods of preparation of bio-based antimicrobial packages

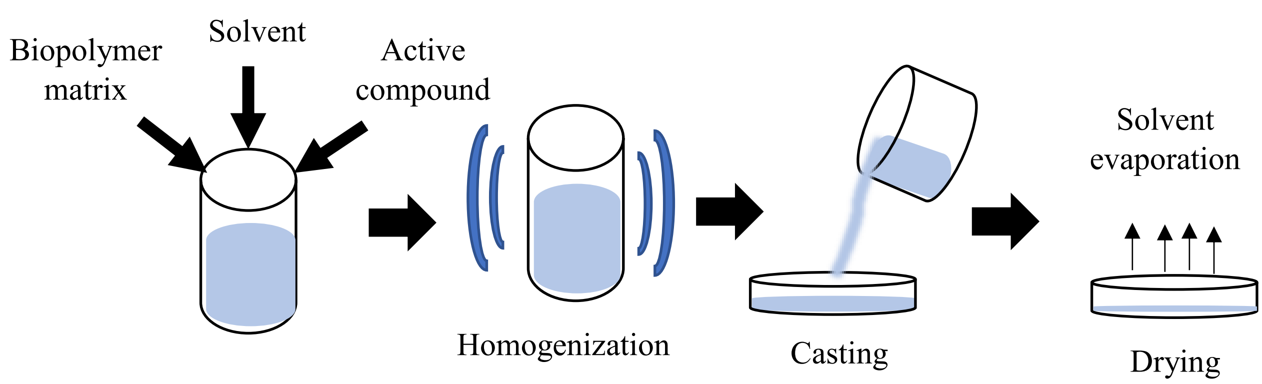
One of the main problems today in the production of bio-based packaging is the determination of the appropriate method for its production at the industrial level. Packaging production has been used for the wet (casting) or dry (thermoforming or extrusion) process for many years. The selection of the production method is crucial because it can change the characteristics of the material. The casting method is a simple process for obtaining edible films. It is regularly used in laboratory studies, but it has limitations in that it formulates films smaller than 25-30 cm and requires 10-24 h of drying. In contrast, dry methods facilitate the production of large-scale packaging at a low cost, which will discuss in more detail in this section (Grumezescu & Holban, 2017; Cerqueira, Pereira, Da Silva Ramos, Teixeira & Vicente, 2017).

3.1 wet process

This process consists of dissolving the biopolymers in suitable solvents, where the additives or functional compounds are added, then these are poured, spread on a surface and dried to evaporate the solvent and thus obtain the film. When drying occurs on the surface of the food, it is called coating or covering. This method is primarily used at the laboratory level because of its simplicity in the equipment required. It has been used to the formation capacity of different bio-based polymers, their physicochemical properties when using other conditions and additives.

The casting method can be synthesized in four steps: dispersion, homogenization, casting and drying (Figure 1.1) (Grumezescu & Holban, 2017; Cerqueira *et al*., 2017). The most commonly used solvents for obtaining films or coatings are water and ethanol. In this technique, higher temperatures can be used for polymer dissolution, as is the case of drying chitosan films using temperatures of 48-58 °C (Kienzele-Sterzer, Rodriguez-Sanchez & Rha, 1982; Cui *et al*., 2017). The method is widely used to prepare bio-based polymer films due to the evaporation of the solvent at room temperature (~25 °C), thus helping to avoid undesired reactions and which may alter the optical characteristics of the films. Some examples of antimicrobial films formed by this method are presented in Table 1.3.

Figure 1.1 Steps of forming films by the casting method



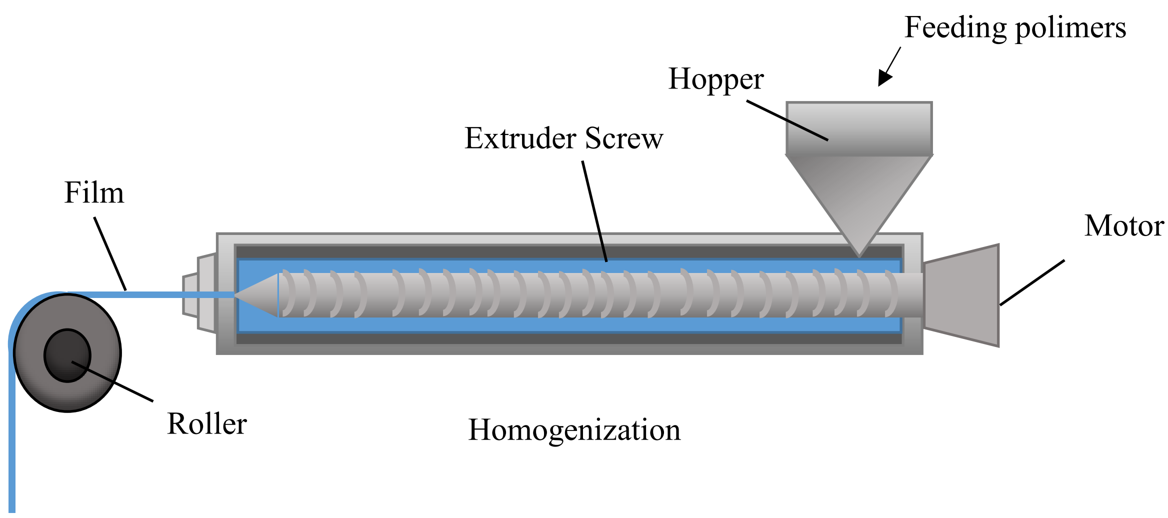
Source: Khan et al. (2018)

3.2 Dry process

The dry process is low cost and is used on an industrial scale. It has been used for materials such as PLA, PHA (polyhydroxyalkanoates), PHB (polyhydroxybutyrate). However, studies for the production of packaging utilizing this technique are minimal. Dry processes include extrusion and thermo-pressing.

Extrusion is a process that uses one or two rotating screws installed in a barrel which increases the pressure, pushes and mixes the ingredients necessary for the manufacture of commercial packaging (figure 1.2). It presents advantages such as a reduction in production time and energy required for solvent removal. However, this process can affect the quality of the films formed by the shear rate and high temperatures of the process (Espitia, Du, Avena-Bustillos, Soares & Mchugh, 2014; Orsuwan & Sothornvit, 2018).

Figure 1.2 Film production by extrusion method.



Source: Pranata et al. (2019)

Colak *et al*. (2015) used the extrusion technique to prepare sodium caseinate and lysozyme films. Their results showed that increasing the time and temperature in the film extrusion process resulted in the loss in lysozyme activity. However, by using a temperature of 65 °C and glycerol concentration of 20-25 %, they retained 26. 4 % of the initial lysozyme activity, a decrease in antimicrobial activity was also reported by Khalid *et al*. (2018), who prepared films using polycaprolactone and starch as matrix and incorporating pomegranate peel as antimicrobial agent. The reduction in antimicrobial activity of the films was attributed to the degradation of pomegranate peel compounds by heating and shearing in the process. Rodriguez *et al*. (2018) studied the effect on the film preparation method of cellulose acetate, triethyl citrate plasticizer, organic clay and cinnamaldehyde. They used the casting and extrusion method for the formation of the films. Their results showed that the extrusion method allowed a better homogenization of the organic clay than the casting method. The degradation of the quaternary ammonium in the organic clay negatively affected the films' color. The antimicrobial activity showed a 25% reduction in the film. This could be caused for using temperatures close to 200 °C that favored the evaporation of the cinnamaldehyde. Despite this loss, the antimicrobial activity tests showed a reduction of 3.5 logarithmic cycles in the growth of *Escherichia coli*, more significant than that observed for the films made by the casting method.

Thermoforming is a method in which high temperatures and pressures are applied to a mixture of polymers with viscoelastic properties that form a film upon cooling. It has hydrophobic, ionic, covalent, and hydrogen bonding interactions that help to stabilize it. This process consists of placing the biopolymer between a pair of thermostated plates that act as a press where it is necessary to regulate the parameters of temperature, pressure, time, type and content of plasticizer and humidity level (Blanco-Pascual & Gómez-Estaca, 2017).

Although thermo-pressing is not a standard method used to prepare antimicrobial films, this method used Moreno *et al*. (2016) to prepare films with corn starch, bovine gelatin, glycerol, and lysozyme. They showed that these films are more permeable to water vapor and oxygen than those prepared by the casting method that they are less rigid and have greater flexibility. The antimicrobial activity of the films obtained showed that the bactericidal activity against *Listeria innocua* is maintained in both methods. Another author who used this method to elaborate antimicrobial films was Valencia-Sullca *et al*. (2018), who formulated films with a mixture of starch, chitosan, glycerol and polyethylene glycol. Their studies showed that, when using a thermal process such as thermo-pressing, it causes a Maillard reaction due to chitosan giving a yellowish appearance to the films.

In general, both processes have advantages and disadvantages in the production of antimicrobial packaging. For example, dry processes are fast methods where less production time and low cost are needed. However, they require specialized machinery and high temperatures that can reduce the activity of the antimicrobial agents at the time of processing. Unlike films produced by wet casting methods, they use low temperatures for the removal of solvents, which helps to maintain the activity of their compounds and require less specialized equipment; however, they are limited to obtaining films of a size of 20-30 cm with prolonged drying times (up to 24 h).

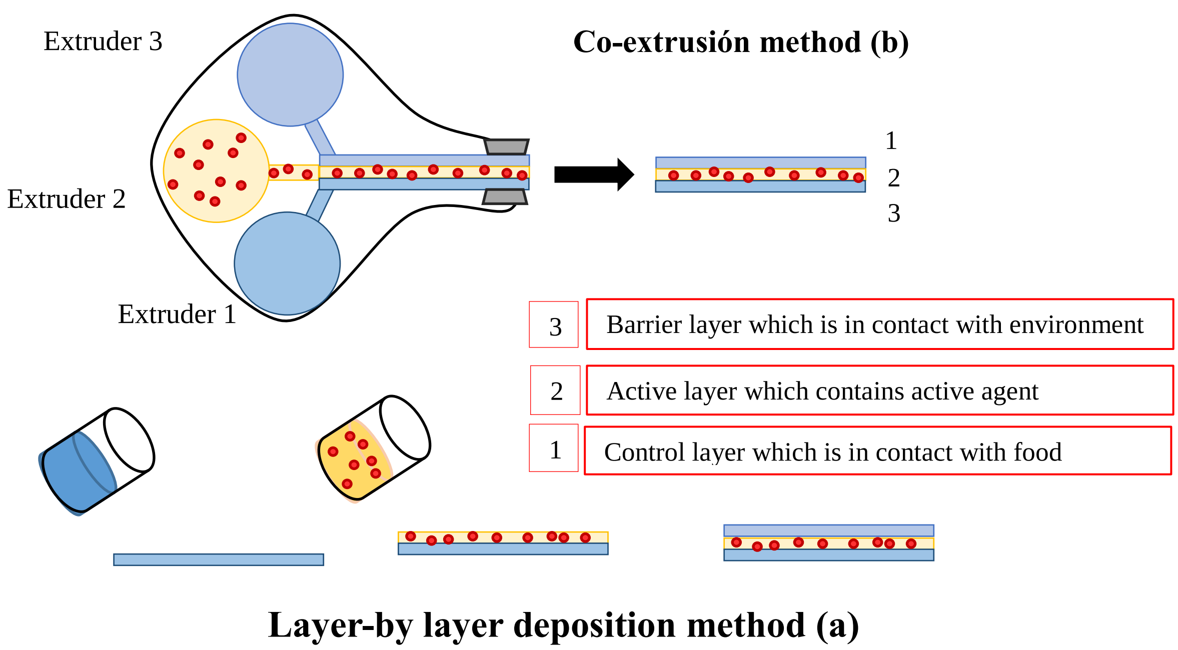
The structure of antimicrobial packaging is also influenced by the type of method used. However, both processes have been used to form unilaminar, bilaminar and multilaminar films that can improve the mechanical and barrier properties of the films.

4. Structure of bio-based antimicrobial packages

The development of bio-based antimicrobial packaging (BBA) is a promising alternative for the substitution of conventional packaging; however, it has disadvantages such as low permeability, which allows the passage of low molecular weight materials that can compromise food quality and safety. Therefore, different studies have proposed different alternatives, such as the combination of polymers or the use of coatings to improve the barrier properties of bio-based packaging without altering its optical and biodegradability properties (Cerqueira *et al*., 2017).

Currently, traditional packaging is rarely manufactured using only one material, especially when talking about flexible packaging. The use of several materials can lead to conform structures that can improve both mechanical and barrier properties, resulting in extending the shelf life of the food. Single or multilayer films containing more than one polymer are used in the industry to improve barrier and mechanical properties, and this type of packaging material has been proposed to control the release of active compounds. Regularly multilayer active films are constituted by three layers, as shown in (figure 1.3.a). They can be elaborated by both wet and dry methods (figure 1.3.b). Thickness, chemical composition and diffusivity are parameters to be considered to design multilayer films (Piergiovanni & Limbo, 2016; Almasi, Jahanbakhsh Oskouie & Saleh, 2020).

Figure 1.3 Methods for the production of multilayer films (a) wet method (casting) (b) dry method (co-extrusion)

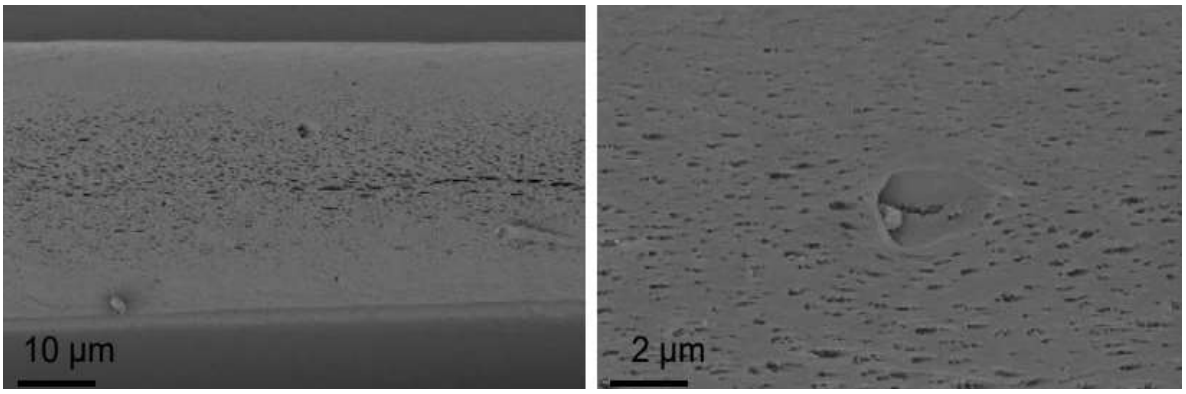


Source: Almasi, Jahanbakhsh Oskouie & Saleh (2020)

Armentano *et al*. (2015) developed antimicrobial films using carvacrol as an antimicrobial agent and combining the polymers PLA (polylactic acid) and PHB (polyhydroxybutyrate). Their results showed that there were changes in the microstructure of the films compared to PLA films where a smooth and uniform surface was shown. In PLA-PHB and carvacrol films, micro-holes (Figure 1.4) were observed on the surface, which could facilitate the release of the antimicrobial.

In terms of mechanical properties, studies showed that the addition of PHB to the PLA matrix did not significantly change the elastic modulus or its elongation percentage. In 2016, Shahmohammadi and Almasi evaluated the morphological, physical and antimicrobial properties of cellulose films produced by *Gluconacetobacter xylinum* strain and ZnO nanoparticles. The film structure was trilayer (two outer layers of cellulose and an inner layer of ZnO and cellulose nanoparticles). The multilayer films showed a smoother surface compared to the unilamellar ZnO and cellulose films. The multilayer film showed lower water vapor permeability and an increase in elastic modulus and breaking point compared to the unilamellar films. A decrease in the antimicrobial activity of the multilayer films was observed in comparison to the free antimicrobial agent incorporated in a monolayer film.

Figure 1.4 Micrograph obtained by field scanning electron microscopy (FESEM) of the PLA-PHB and carvacrol film



Source: Armentano et al. (2015)

Wang *et al*. (2019) formularized and characterized multilayered films of chitosan, sodium alginate and carboxymethyl chitosan. The data obtained on the surface morphology of the multilayered film showed that added ZnO formed aggregates. The solubility and water vapor permeability decreased in the multilamellar films compared to films made only with chitosan and chitosan-alginate. For the antimicrobial activity, the results showed that the addition of ZnO improved the activity of the multilamellar film depending on the proportion in which it was present, also showed that there was an inhibition of the chitosan unilamellar films against *Staphylococcus aureus* and *E. coli*.

As mentioned above, the final properties of the antimicrobial films are modified by the method and the type of structure they present. Another parameter that affects the properties of the packaging and that has to be considered is the choice of the materials used for its elaboration. Among the main components used in the manufacture of antimicrobial packaging are the polymeric matrix, the antimicrobial agent and the type of plasticizer used. The latter can be defined as a low molecular weight volatile compound that is added to polymers to reduce brittleness and impart flexibility. Plasticizers reduce intermolecular forces and increase the mobility of polymer chains, reducing the glass transition temperature of films and improving their flexibility and elongation. Commonly used plasticizers for film and coating production are monosaccharides, oligosaccharides, polyols, lipids and derivatives (Sothornvit & Krochta, 2005; Azeredo *et al*., 2011).

The type of plasticizer used will depend on the polymeric matrix used for the formation of the container, for which the characteristics of each of these polymers, their compatibility and possible interactions must be taken into account. In the following section, some studies and characteristics of the main polymeric matrices studied will be discussed in general terms.

5. Biopolymers used in antimicrobial packaging

Biopolymers have been studied as an alternative to traditional polymers used in food packaging; however, their implementation is not limited to packaging, as they can have applications in medical materials, cosmetics, food additives, water treatment chemicals, and absorbents, among others. Biopolymers are produced by living organisms and can be derived from microbial systems, extracted from plants, or chemically synthesized.

These biodegradable materials can be classified into four large families: the first are polysaccharides that can include starch, cellulose and chitosan; the second family are proteins such as gluten and zein; the third includes the use of oils; and finally, polymers that are produced by natural or genetically modified microorganisms where we can find PHA (polyhydroxyalkanoates) and PHB (polyhydroxybutyrate). Among the most studied characteristics of these polymers when forming films, we can find their barrier and mechanical properties (Rebelo, Fernandes & Fangueiro, 2017; Zhao *et al.*, 2019).

The barrier properties of materials indicate their resistance to diffusion and sorption of substances, a polymer with suitable barriers has low diffusion and solubility coefficients. The diffusion coefficient measures the speed with which the substance penetrating the polymer could move within the polymer matrix. On the other hand, the solubility coefficient gives us the concentration of the substances absorbed by the polymer upon contact with it. Another term used to measure barrier properties is the permeation coefficient, which combines diffusion and solubility coefficients. Permeation is the ability of a permeant to penetrate and pass through material in response to the difference in partial pressures. The barrier characteristics of a polymer are commonly associated with its permeability coefficient values, so low permeability values are found in polymers that allow the low mass transfer, the lower this value, the lower the transfer of the type of fluid being evaluated, for example, in food packaging permeability to water vapor and oxygen are commonly evaluated (Culter *et al.*,2016; Han, 2005).

The mechanical properties of polymers show us their behavior, and these depend on the type of polymer and the additives used. It is necessary to use a force-strain curve to examine the mechanical behavior of packaging materials. This curve shows the force required for deformation to occur. The curve helps us obtain the elastic modulus or Young's modulus, which indicates the ratio between the applied stress and the force produced in the elastic portion of the material's behavior and is found in the linear part of the curve. As this parameter increases, the amount of stress required to achieve a given deformation increases, which means a stiffer material. After the modulus of elasticity, a point known as the elastic limit is reached, which is the maximum stress that the material can withstand, where the deformation that occurred disappears, and the material returns to its original dimension. Beyond this point, we find the plastic region where the material begins to increase in deformation without increasing the required stress. Finally, tensile strength is the maximum resistance of a material subjected to a tensile load (Culter *et al.*,2016).

5.1. Polysaccharides

Polysaccharides are the most studied biopolymers due to their biocompatibility, biodegradability and non-toxicity. This large group of macromolecules is classified according to their structure, chemical composition, application and origin. The latter is the most common and classified into those extracted from plants, algae, lichens, and other bioactive polysaccharides derived from animals. Polysaccharides are used to elaborate edible films or coatings in the packaging area due to their hydrogen bonds that form a network. Polysaccharides present good barrier properties against oxygen. However, they have a poor barrier against humidity due to their hydrophilic nature. Polysaccharides are used in packaging to prolong the shelf life of fruits, vegetables, seafood, or meat products because they reduce dehydration, oxidative rancidity, and browning on the food surface. Cellulose, chitosan and starch are some of the most representative polysaccharides (Liu, Willför & Xu, 2015; Hassan *et al*., 2018).

5.1.1. Cellulose

Cellulose is the most abundant polymer among organic compounds and is made up of glucose units linked by β-1,4-glycosidic bonds. The most commercially exploited source for extracting cellulose is wood. However, it can also be found in plants, algae and those produced by bacteria. It is a crystalline liquid with high strength, flexibility, biocompatibility and biodegradability. Its stability is due to the numerous hydroxyl groups present in its structure that lead to forming a network with intramolecular hydrogen bridge bonds. Cellulose is insoluble in water due to the relatively long length of its constituent cellulose chains and the closeness of their hydrogen bonding. Solvents such as ionic liquids, NaOH solutions, among others, are regularly used for film production. Films made from this polysaccharide tend to show advantages by being tasteless, flexible, odorless, transparent, resistant to fats and oils, have hydrophilic nature and have low oxygen and moisture diffusion (Piergiovanni & Limbo, 2016; Karaki *et al*., 2016; Cazón *et al*., 2017; Mohamen *et al*., 2020).

The most commonly used cellulose derivatives are carboxymethylcellulose, methylcellulose and hydroxypropylmethylcellulose. These present a low barrier to water vapor due to their hydrophilic characteristics. The incorporation of different compounds has been studied to counteract the adverse effects of cellulose derivatives on their barrier properties. Saringat, Alfadol, and Khan (2005) studied the effect of polyethylene glycol and triacetin in hydroxypropyl methylcellulose coatings and observed that the addition of the plasticizer resulted in coatings with lower resistance to attraction and increased water vapor permeability compared to the control. In contrast to the addition of triacetin that decreased water vapor permeability. On the other hand, De Melo Fiori *et al*. (2019) combined carboxymethyl cellulose with polyethylene glycol and sodium-based clay nanofillers. The results showed that the addition of the clay improved the mechanical properties by increasing their strength, elastic modulus and elongation percentage and significantly decreased the water vapor permeability of the films.

5.1.2. Starch

Starches are made up of α-ᴅ-glucose units, mainly containing 20-30% amylose (straight-chain polymer) and 70-80% amylopectin (glucose polymer having a branched-chain structure). Starch can be found in wheat, rice, corn, tapioca and potato and is a non-toxic, low molecular weight and renewable material. Another advantage it presents is the formation of transparent films, without color, odor and taste, important characteristics in food packaging (Karaki *et al*., 2016; Hassan *et al*., 2018; Mohamen, El-Sakhawy & El-Sakhawy, 2020). The films or coatings made with starch usually have good barrier properties to CO_2 and O_2. However, Their hydrophilic characteristics, soluble in water and have a poor barrier against water vapor.

Starch film formation starts with the heating of starch granules and water to form a viscous solution. Excess water and high temperatures cause the starch to transform from a semi-crystalline state to an amorphous state, known as gelatinization. This transition process depends on the amylose-amylopectin ratio, water content and dispersion temperature. After gelatinization, the retrogradation process allows the amylose and amylopectin chains to be dissociated in a starch dispersion to reassociate into an ordered structure that gives starch films their final permeability and mechanical properties (Thakur *et al.,* 2019).

The elaboration of films from this polymer has varied according to its components and structure. Ghanbarzadeh, Almasi and Entezami (2010) elaborated films of starch and carboxymethyl cellulose (CMC). Their results showed that the addition of CMC in starch films reduced water permeability. When they increased to 20% CMC, the tensile strength increased to 59% more than pure starch. Concerning color, the films showed a decrease in yellowness values and an increase in lightness. In 2013, Das *et al*. studied the effect of a coating of rice starch with coconut oil and tea leaf extract on tomatoes. Their results showed that the weight loss in coated tomatoes was less than that of the control. It also showed a delay in the ripening effect on the tomato, which extended its shelf life.

Another study combining the properties of starch with different materials is Saberi *et al*. (2018), who developed multilayer films using pea starch, guar gum and a mixture of lipids. The addition of lipids in the starch films decreased the fruit respiration rate, ethylene production, firmness and weight loss, peel peeling, and decay rate of orange fruit, thus extending the fruit's shelf life. Starch films added with antimicrobials have also been used to inhibit the growth of pathogenic microorganisms in ready-to-eat foods. Zhao *et al*. (2019) formulated films with cassava starch, carvacrol, chitosan and gallic acid to inhibit the growth of *Listeria monocytogenes* in ham slices.

5.1.3. Chitosan

It is a polysaccharide that has been widely studied for the formation of active films for its antimicrobial and antifungal activity. Like other polysaccharides, it has low permeability to water vapor and oxygen. It is extracted from chitin, which is present in crustaceans, mollusks, insects, algae and related organisms. Approximately 100 billion tons are produced annually from these sources, making it the second most abundant polysaccharide after cellulose. Chitin consists of N-acetyl-ᴅ-glucosamine chains linked by β-1,4-glycosidic bonds. To obtain chitosan, chitin has to go through chemical or enzymatic processes, being the chemical one the most used due to its low cost and capacity for mass production. Chitosan is obtained from the deacetylation of chitin, formed by ᴅ-glucosamine and N-acetyl- ᴅ-glucosamine units with β-1,4 glycosidic bonds.

When the degree of deacetylation is approximately 50 %, chitosan becomes soluble in acidic media, however, its solubility will depend on the degree of N-acetylation and its molecular weight. (Chang, Tsai, Lee & Fu, 1997; Tripathi, Mehrotra & Dutta 2008; Cazón *et al*., 2017; Muxika *et al*., 2017; Domínguez *et al*., 2018).

The formation of chitosan films has been done by varying different parameters in their preparation, such as the type of plasticizer, the drying method and the addition of antimicrobial agents. In 2005, Suyatma et al. observed the effect of four different plasticizers (glycerol, ethylene glycol, polyethylene glycol and propylene glycol) on the mechanical properties of chitosan films and their storage stability. The use of glycerol, ethylene glycol, polyethylene glycol improved the ductility of chitosan films, however, propylene glycol made the films more brittle. They found that, over time, most of the films showed a decrease in their percentage elongation. They concluded that glycerol and polyethylene glycol were the most suitable because of their storage stability. Regarding the effect of drying type and incorporation of antimicrobial agents, Thakhiew, Devahastin and Soponronnarit (2013), studied the effect on the concentration of active agent and drying method (hot air and low pressure superheated steam drying) had no significant effect on water content, the thickness and water vapor permeability of chitosan and blue ginger films, but had influence on the color, degree of crystallinity, attractive strength, elongation percentage and oxygen permeability.

5.2. Proteins

Proteins are presented in fibrous or globular. They function as structural materials of tissues and perform different functions in living systems. The physicochemical characteristics of proteins depend entirely on the arrangement of amino acid substituents and the concentration in which they are present along the polymeric chain. They have good mechanical and optical properties while providing a suited barrier against aromas, oxygen, organic vapors and selective permeability to other gases. Despite these advantages, films made from proteins can be affected by their high moisture content. Different types of globular proteins have been proposed for film or coating formation, such as wheat gluten and corn zein (Dominguez *et al*., 2018; Hassan *et al*., 2018).

5.2.1. Corn Zein

Zein is a prolamin and the main protein in corn. It is hydrophobic and a thermoplastic material because it is strong, shiny, bacteria-resistant, water-insoluble, antioxidant, and adhesive. It can be dissolved in 70-80 % ethanol, high concentrations of urea, alkalis or anionic detergents. It contains high concentrations of amino acids such as glutamic acid, proline, leucine and alanine. In the food industry, zein is used as a coating material for candies, fresh and dried fruits, nuts, and incorporation into chewing gum. Zein films are usually formed by drying the medium in which they were dissolved, and the use of plasticizers is essential to give the films flexibility as they are brittle. Due to its excellent gas and moisture barrier properties, it is a good alternative for film production. There are several studies on the elaboration of zein films and antimicrobial agents in the literature and the possibility of producing them using thermo extrusion methods. However, it has been seen that this method is not compatible since some biopreservatives lose their activity during the thermal process (Yemenicioglu, 2016; Aguirre- Joya *et al*., 2018; Hassan *et al*., 2018).

These films have been developed in recent years by combining them with antimicrobial agents and different biopolymers to modify their structural characteristics. Vahedikia *et al*., in 2019, incorporated cinnamon essential oil and chitosan nanoparticles into the polymeric matrix and observed that it significantly improved the tensile strength and decreased the elongation percentage of zein films. They also determined a higher crystallinity in the presence of cinnamon essential oil and chitosan nanoparticles. The films showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Another study elaborated with Zein active films was that of Haiying Cui *et al*. in 2020, who added pomegranate peel extract encapsulated in chitosan nanoparticles to zein films. They also applied liquid nitrogen plasma to modify the surface of the films to maintain the release of pomegranate polyphenols from the nanocomposite films. Their results showed higher thermal stability of these films compared to those developed with pure zein alone. The release of the active agent was lower in the plasma-treated films and significantly inhibited the growth of *Listeria monocytogenes* compared to the untreated and control films.

5.2.2. Wheat gluten

Wheat gluten is a globular protein that combines polypeptide molecules. Its properties, such as cohesion and elasticity, facilitate the film formation process. This protein plays an essential role in bread making, just as zein is insoluble in water. The formation of gluten films depends mainly on breaking its disulfide bonds during the thermal process and on the formation of new disulfide bonds during the drying of the films. In addition to these changes, the hydrogen bonds also undergo modifications. The surface of gluten films regularly is glossy, has good insulation to oxygen and limited resistance to water vapor (Micard *et al*., 2000; Aguirre- Joya *et al*., 2018; Hassan *et al*., 2018; Chen *et al.* 2019).

According to studies by Gontard *et al*. (1992), when testing different ethanol concentrations (70-20 mL/ 100 mL) and pH (2-6) in gluten film-forming solutions, an effect on opacity, solubility and water vapor permeability was observed. Permeability can be modified mainly by gluten concentration and pH. As with the other polymers, the preparation and the addition of plasticizers determine the final characteristics of the films. According to the studies of Mangeavel *et al*. (2004), the type of method by which gluten films were prepared affected the tensile strength and elongation percentage. Their results showed that the tensile strength was higher for films formed by thermo-pressing compared to those formulated by the casting method (4.15±0.78 MPa and 0.60±0.15 MPa, respectively) but showed lower values of elongation percentage (179±46% and 732±90%). Regarding the influence of the plasticizer, in both cases, if the plasticizer concentration increased, the value in tensile strength decreased.

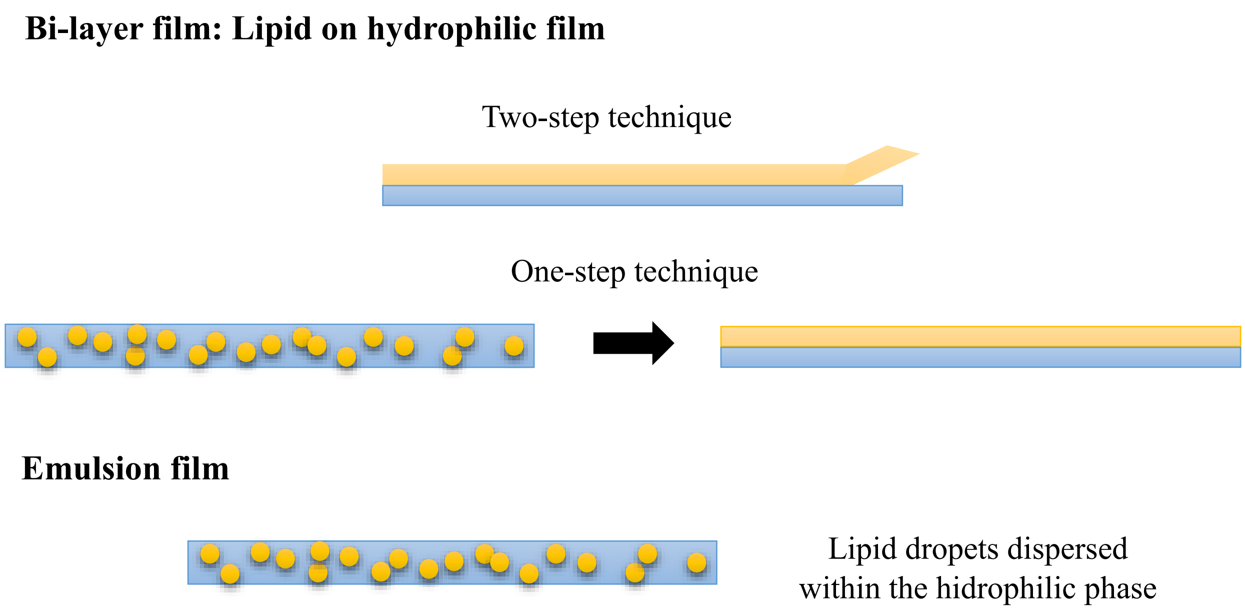
Gluten films added with bacteriocins have also been formulated to inhibit *Listeria innocua*. According to Blanco Massani *et al*. (2014), the addition of 0.1 % Lactocin 705 and lactocin AL705 inhibited the growth of *Listeria innocua* 7 and *Lactobacillus plantarum* CRL691. In other studies, in 2015, El-Wakil *et al*. added TiO_2 nanoparticles and cellulose nanocrystals in wheat gluten films and studied their effect on tensile strength, elastic modulus, and water vapor permeability. They found that the films changed their percentage elongation using 0.6 and 0.8 % TiO_2 and 7.5 % single cellulose crystals. The films with cellulose modified the water vapor permeability, obtaining lower values than films made only with gluten. The addition of TiO_2 also showed a reduction in water vapor permeability in films with 7.5 % cellulose.

5.3. Lipids

Lipids are found in sources such as plants, animals and insects, and for several years have been used to make edible films and coatings used in the food area to preserve fruits and vegetables. Lipids reduce water vapor permeability due to their hydrophobic nature and provide gloss. However, they form brittle and thick films. Their poor mechanical properties mean that they have to be combined with biopolymers such as proteins and polysaccharides. They can be associated in the form of emulsions or bilamellar films. In the emulsion system, the lipid is mixed in the film-forming solution and then formed by the casting method, regularly stabilized with an emulsifier, so that phase separation does not occur and a monolayer film is obtained. In the layer-by-layer system, the same way as the unilamellar films, the lipids are cast on the previously dried layer of the biopolymer.

Figure 1.5 illustrates these two procedures. Their efficiency as a barrier to water vapor depends mainly on the nature of the lipid, the length of the fatty acid chain and the structure of the emulsion that constitutes the dry film. This group comprises monoglycerides, diglycerides, triglycerides, cerebrosides, phosphatides, phospholipids, terpenes and fatty acids. Due to their greater efficiency as a moisture barrier, animal or vegetable waxes are the most widely used in preserving fruits and vegetables (Debeaufort *et al.*, 2000; Han, 2005; Cerqueira *et al.,* 2017; Aguirre-Joya *et al*., 2018).

Figure 1.5 Formation of films composed of a lipid and a biopolymer



Source: Han (2005)

5.3.1. Waxes

Waxes are esters of long-chain fatty acids with long-chain alcohol, non-polar, high hydrophobicity, insoluble in aqueous media and soluble in organic solvents. They are usually moldable at room temperature, brittle and do not show good elasticity. Waxes are soluble in hexane, chloroform or benzene. These molecules have no polar constituents or have such a small hydrophilic part that they cannot interact with water, thus preventing the molecules from diffusing, which may explain their efficiency as a barrier to water vapor. Waxes are found in living organisms, such as in bird feathers that repel water, and it has also been observed that some invertebrates produce waxes to keep their skin lubricated and repel water. They are also found on the surface of leaves and fruits. This group can include natural waxes such as carnauba wax, candelilla wax, rice bran wax, and beeswax (Han, 2005; Yurcanisn Bruice, 2007; Wade, 2011; Robersont, 2013; Aguirre- Joya *et al*., 2018).

Waxes can be used for food preservation, such as the films developed by Oregel-Zamudio *et al*. (2017), who combined candelilla wax films with *Bacillus subtilis* strain HFC103 to preserve strawberries. Treatment of strawberries with these films showed a 100 % reduction in fruit decay relative to the control on the sixth day of storage. And a reduction in the severity index, which indicates the damage caused by the presence of mold in fruit, was reported on days 2,4,5 with percentages of 21,41,47, 54 and 56 %, with respect to the control. Other studies conducted by Aguirre-Joya *et al*. (2019) used candelilla wax, pectin, aloe vera mucilage, glycerol, and Larrea tridentata leaf extract to produce a coating. They showed that it reduced the damage caused by *Colletotrichum gloeosporioides* to 22 % and *Alternaria alternata* to 24.5 % in avocados. By 2018, Motamedi *et al*. used a combination of nanoclay and carnauba wax to preserve the quality of "valencia" orange, showing that the application of this coating improved fruit acceptability, nutritional quality and reduced fruit weight loss during storage.

5.4. Polymers synthesized by microorganisms

Biopolymers that are synthesized to aid the survival and function of microorganisms can occur intracellularly, structurally and extracellularly. Intracellularly accumulated polymers, as granules within the cytoplasm of cells, have mechanical properties similar to elastic rubber or crystalline hard plastic. Exopolymers can occur as slime or encapsulate that can be separated from the medium by centrifugation. Microbial biopolymers perform specific functions as a source of energy or as protective agents. They are also released to help microorganisms function, adapt, multiply and survive. They have a wide variety of functions in food, medicine and other applications. They are neutral or acidic in nature. Some produce very high viscosity in aqueous solutions, and others form gels similar to agar and carrageenan. Various physical and chemical parameters influence the production of these biopolymers.

Their low production cost makes them an attractive alternative Table 1.2 presents some microorganisms producing these biopolymers where bacterial cellulose, kefiran, pullulan, gellan and xanthan are produced extracellularly, and PHA is found in those produced intracellularly (Vijayendra & Shamala, 2013; Singh *et al*.,2015).

Tabla 1.2 Examples of polymer-producing microorganisms

Biopolymer	Microorganisms	Reference
Bacterial cellulose	*Acetobacter sp.* *Rhizoium sp.*	Shoda y Sugano, 2005 Castro *et al.*, 2012
Kefiran	Microflora kefir	Kooiman, 1968
Pululano	*Aureobasidium pulluslans*	Goksugur *et al.*,2011
Gellan gum	*Sphingomonas paucimobilis* *Pseudomonas elodea*	Bajaj *et al.*, 2007 Banik & Santhiagu, 2006
Xanthan	*Xanthomonas campestris*	Kalogiannis *et al.*, 2003; Fitzpatrick *et al.*, 2013
Polyhydroxyalkanoates	*Bacillus sp.* *Chelatococcus sp.*	Vijatendra *et al.*, 2007 Divyashree *et al.*, 2009
Polylactic acid from lactic acid bacteria	*Lactobacilus bulgaricus* *Lactobacilus delbrueckii*	Inquinen *et al.*, 2011 Lasprilla *et al.*, 2012

5.4.1 Polyhydroxyalkanoate (PHA)

Hydroxylalkanoates are bioplastics produced by microorganisms, which are accumulated into granules intracellularly by a wide variety of microorganisms in the presence of a carbon source and a limited supply of nutrients such as nitrogen, phosphorus or oxygen. PHAs are the only family of polymers that act as a carbon or energy source for more than 300 Gram-positive and Gram-negative bacteria species. They have hydrophobic characteristics, so they are insoluble in water. It is a thermoplastic and elastomer, non-toxic, resistant to UV degradation, and pure within the cell. By their chain length can classify PHA into short-chain (3-5 carbons), medium-chain length (6-14 carbons), and long-chain length (more than 15 carbon atoms). The elastic modulus presented by this polymer can vary from 0.008 MPa to 3500 MPa, its elongation percentage has been reported to vary from 2% to 1000%, and its tensile strength generally varies from 8.8 to 104 MPa. Several derivatives have been studied within the PHA group that can count with permeabilities very similar to synthetic polymers such as polyvinyl chloride and polyethylene terephthalate (Laycock *et al*., 2013; Angelina & Vijayendra, 2015; Masood, 2017; Meereboer, Misra & Mohanty, 2020).

Polyhydroxybutyrate (PHB) is one of the most produced PHAs and was discovered by Lemoigne in 1927. In 1923, Lemoigne characterized PHB chemically and observed that it was associated with the sporulation of *Bacillus* spp. This is associated with lipids accumulated by various bacteria as they enter their stationary growth phase and then used as an internal reserve carbon and energy source. PHBs commonly accumulate in response to essential nutrient restrictions, so past studies for the synthesis of this polymer have focused on growth stress conditions (Page, 1995).

Studies on active films with PHB may include incorporating essential oils and other compounds to inhibit bacterial and fungal growth. Narayanan *et al*. (2013) incorporated eugenol at a ≥40 mg / g PHB concentration for bacteria and ≥80 mg / g PHB for fungi in PHB films produced by *Bacillus mycoides* strain DFC1. The films showed inhibition against *Staphylococcus aureus* MTCC 737, *Salmonella Typhimurium* MTCC 98, *Escherichia coli* NCIM 23058, *Bacillus cereus* MTCC 1272, *Aspergillus flavus* MTCC 277, *Aspergillus niger* MTCC 162 and *Penicillium* sp. MTCC 4610. These results agree with those reported by Xavier *et al*. (2015), they formulated PHB films produced by *Bacillus mycoides* DFC1 (isolated from garden soil) and added vanillin as an antimicrobial, and inhibited at a concentration of ≥80 µg/ g PHB for bacteria and ≥50 µg/ g PHB for fungi. The films showed activity against *Escherichia coli*, *Salmonella Typhimurium*, *Shigella flexneri* and *Staphylococcus aureus*. Also showed activity against fungi such as *Aspergillus*, *Aspercegium parasites*, and *Penicillium clavigerum*.

Rech *et al*. (2020) developed films of PHB and essential oil of cinnamon, melaleuca and citronella, having an inhibitory effect against *Aspergillus niger*. The addition of essential oils increased the degree of crystallinity and stability of PHB films. However, it had a plasticizing effect on the films, reducing the polymer's melting temperature and giving it greater flexibility. As explained in this section, biopolymers have different origins.

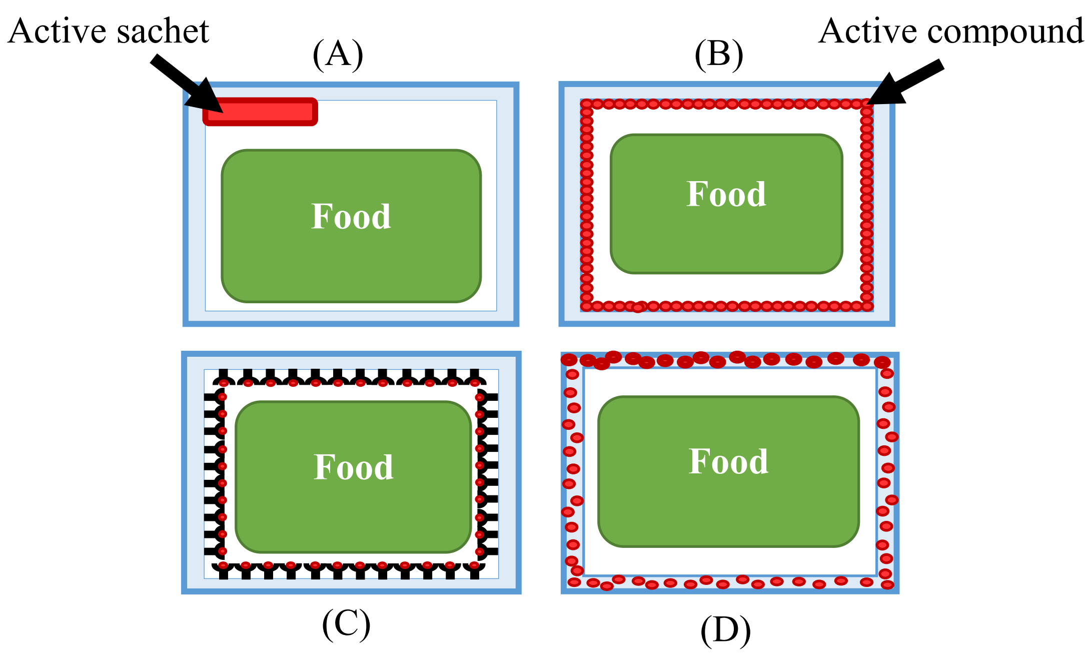
They are found in plants, animals, insects and can even be synthesized by microorganisms. Their properties and characteristics are helpful for food preservation. In the research carried out in the last years, it has been sought not only the individual characterization of the polymer but also the combination between them, with plasticizers and in particular with antimicrobial agents that modify their activity against pathogens present in food. The incorporation and composition of the active agent can also alter the structure of the packaging developed, so it is important to discuss them.

6. Main active agents used in antimicrobial films

The antimicrobial activities of packaging are based on the migration of antimicrobial substances from the package to the food. These substances inhibit or reduce the growth of microorganisms, retains its desired qualities and increasing shelf life. However, there are also packages where there is no migration of the active compound. Some designs of active agent incorporation systems are shown in Figure 1.6.

In Figure 1.6 (A), the active agent is incorporated in a small bag between the packaging and the food. This system is usually used for oxygen and moisture-absorbing agents. In the case of Figure 1.6 (B), a system is presented where the active agent covers the inner surface of the package. It is used for heat-sensitive or incompatible and immiscible compounds with the polymeric matrix. On the other hand, in the system shown in Figure 1.6 (C), the active agent is immobilized on the packaging surface by ionic or covalent bonds that prevent release to the food. Finally, in Figure 1.6 (D), the antimicrobial agent is added to the film-forming mixture. This system presents advantages such as uniform distribution in the polymeric matrix, high resistance to processing conditions and slow release into the food. This last design allows the efficient migration of the antimicrobial, which acts on the cells slowly and gradually, allowing its activity to remain for a prolonged time (Nerin *et al*., 2016; Alsami *et al*.,2020).

Figure 1.6 Active food packaging system designs



Source: Almasi, Jahanbakhsh Oskouie & Saleh (2020)

Note: (A) use of active sachet inside the packaging, (B) coating of an active agent on the polymer, (C) immobilization of active agents on the polymer surface, (D) incorporation of the active agent into the polymer matrix

6.1. Enzymes

Enzymes have many applications in food. They can be added directly to the food or incorporated into films. Lysozyme is an enzyme that sensitive bacteria by breaking down peptidoglycan polymers found in cell walls. Its activity isn't limited only to bacteria as it shows activity against fungi, viruses and protozoa. Lysozyme activity is limited to Gram-positive bacteria as the cell wall components give free access to the enzyme, whereas, in Gram-negative bacteria, the lipopolysaccharide layer of the outer layer is a barrier against lysozyme attack (Mousavi Khaneghah *et al*., 2018).

The utilization of enzymes, especially lysozyme, has been reported for application in developing active films. Fraba, Sanchez-Gonzalez and Chiralt (2014) incorporated lysozyme in two different polymeric matrices (corn starch and pea proteins) and studied the enzyme release at 10 °C and 25 °C. Both films had activity against *Listeria monocytogenes* at a temperature of 10 °C. However, this activity was affected at 25 °C, where pea protein films showed higher activity by reducing pathogen growth by 40 % compared to the control. In 2015, Kaewprachu *et al*. developed catechin-lysozyme gelatin films to maintain the quality of ground pork, compared their preservation efficiency against polyvinyl chloride (PVC) films during storage (7 days at 4 °C). They observed less weight loss and minor discoloration in the lysozyme-catechin films than those wrapped with PVC. Regarding antimicrobial activity, the growth of microorganisms on catechin-lysozyme gelatin films was 4.15 ±0.72 log CFU/g at 7 days of storage, lower than that reported for PVC films that reported growth of 5.87±0.31 log CFU/g.

Khairuddin *et al*. 2017, made an antimicrobial film with wheat gluten, lysozyme and ethylenediaminetetraacetic acid as antimicrobial agents. A reduction of *Escherichia coli* and *Bacillus subtilis* growth to 1.74 and 3.48 log CFU/mL was observed. Wu *et al*. (2018) developed chitosan and lysozyme coatings. They evaluated the effect on the quality of yellow croakers. Films with lysozyme presented growth of 5.86±0.40 log CFU/g, at 15 days of storage, values lower than the maximum allowed (7.0 log CFU/g). Lipid oxidation in chitosan films with or without lysozyme showed a reduction in the thiobarbituric acid index and improved sensory evaluation scores.

6.2. Bacteriocins

Bacteriocins are antimicrobial peptides synthesized as metabolites of lactic acid bacteria. They are active against a large number of microorganisms. Bacteriocins are varying in size, structure and specificity. The mechanism of action of bacteriocins is through interaction with cell membranes causing their death. It is an antimicrobial agent investigated for its natural characteristics, which does not modify food's sensory properties. Its incorporation in films has been of great interest due to its activity against Gram-positive bacteria and its resistance to high temperatures and acidic media. Nisin and pediocin are the most widely used in developing active packaging. However, studies aren't limited to these two bacteriocins (O'Connor *et al*., 2015; Martinez, Rodriguez & Suarez, 2016).

Woraprayote *et al*. (2013) incorporated pediocin PA-1 into polylactic acid (PLA) films and sawdust particles by diffusion method. In vitro testing of the films was performed on pork slices using *Listeria monocytogenes* ATCC 19115 as a sensitive strain. The results showed an zone of inhibition of the films from 2.75 to 3.88 mm for PLA films, sawdust particles and pediocin. However, PLA films alone with bacteriocins did not show inhibition against this pathogen. They considered that most probably the sawdust particles promoted the adsorption of the bacteriocin on the films, which increased their efficacy. In pork samples, the films reduced the *Listeria* population by 1.5-2.0 log cycles. Another study using this incorporation method was Woraprayote *et al*. (2018), who impregnated films of polylactic acid and sawdust particles with bacteriocin 7293 produced by *Weissella hellenica* BCC 7293. The films were prepared by extrusion. The maximum concentration absorbed by the films of bacteriocin 7293 was 19.54 μg/cm^2. Its activity was tested against Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Escherichia coli* and *Salmonella typhimurium*) that are responsible for the rejection of pangasius fillets. The tests showed inhibition for Gram-positive and Gram-negative bacteria in both in vitro and pangasius fish fillets.

In addition to the use of bacteriocins for film production, they have been used to make coatings. Guitian *et al*. in (2019) added enterocin produced by *Enterococcus avium* DSMZ17511 to develop antimicrobial coatings with food-grade agar. The coatings were used to wrap cheeses with low moisture content and artisanal cheeses with high moisture content. The cheese samples were previously inoculated with *Listeria monocytogenes* 01/155. Results showed a reduction of pathogen growth by 1.0-1.5 log cycles compared to control by day 8 and a 2.0 log cycle reduction by day 10 of storage. In artisanal goat cheeses, the reduction of *Listeria* was 5 log CFU/mL after two weeks of testing.

6.3. Essential oils

They consist of volatile compounds such as terpenoids, aliphatic chemicals and terpenes. They are derived from plants and have low molecular weight. Essential oils are containing phenolic compounds such as thymol, eugenol and carvacrol show higher antimicrobial activity against all types of microorganisms (Sanchez-Ortega *et al*., 2014). The use of essential oils in films has been widely studied as they can be extracted from natural sources such as plants.

The activity of a single essential oil can vary depending on the strain tested, as observed in studies done by Hafsa *et al*. (2016), who tested the antimicrobial activity of chitosan films with Eucalyptus globulus essential oil. The films showed a zone of inhibition from 54.53 to 153.37 mm^2 for *Escherichia coli*. *Pseudomonas aeruginosa* showed a smaller zone of inhibition ranging from 27.80 to 118.29 mm^2. In the case of *Staphylococcus aureus*, they showed zones from 10.56 to 61.35 mm^2. Finally, the films showed inhibition zones to *Candida parapsilosis* from 8.43 to 65.94 mm. This strain is the most resistant to this essential oil. In 2017, Kashiri *et al*. extracted essential oils from Zataria multiflora Boiss, a plant that grows in central and southern Iran, and incorporated it into zein films that were subsequently applied on the surface of polypropylene bags. Milk previously inoculated with *Listeria monocytogenes* and *Escherichia coli* was packaged in these bags and stored at 4 °C for 6 days. The packages with the essential oil showed a reduction of 0.65 log CFU/mL on day 1, 1.10 and 0.91 log CFU/mL on day 3 and 6 for *Listeria*. In the *Escherichia coli* strain, the reduction in growth was 0.51, 0.92 and 0.99 log CFU/ ml on days 1, 3 and 6.

Iamareerat *et al*. (2018) formulated cassava starch films with cinnamon essential oil and sodium bentonite. They observed that as the essential oil concentration increased, the zone of inhibition increased of the sensitive strains studied: *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. The highest inhibition was observed at a 2.5 % cinnamon essential oil concentration, with a halo of 15.25 mm for *Escherichia coli* and 10.75 mm for *Staphylococcus aureus*. The films were also tested on pork meatballs. The films maintained the microorganisms' growth in these samples below the permitted values (10^6 CFU/g) for 48 h at 25 °C.

The effect of combining essential oils with another active agent has also been studied. Arezoo *et al*. (2019) analyzed the effect of incorporating titanium dioxide nanoparticles and cinnamon essential oil in sago starch films. In vitro tests were performed using *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. The results showed a higher inhibition on gram-positive bacteria than gram-negative bacteria. These could be caused because Cinnamaldehyde has a hydrophobic nature that helps to destabilize and rupture the membrane of the bacteria, and when combined with TiO_2, leads to cytoplasmic leakage, which causes the death of the bacteria.

7. Effects of antimicrobials on mechanical and barrier properties

The addition of active compounds in polymeric matrices favors the activity and growth control of microorganisms. Research on their mechanical and barrier properties has shown that their incorporation alters their structure, modifying their final characteristics. Incorporating an active agent could increase the barrier properties or permeability to the water vapor of films that depend on the active agent's type of interaction in the polymeric matrix.

Xavier *et al*. (2015) showed that the incorporation of Vanillin in PHB films presented an increase in the elongation percentage, from 0.91% for pure PHB films to 2.09% for films with vanillin making them more elastic. Kaewprachu *et al*. (2015) observed an increase from 2.61 to 4.25×10^{-6} g mm h^{-1} cm^{-2} pa^{-1} in water permeability by incorporating catenin and lysozyme in gelatin films compared to polyvinyl chloride films. Solubility was also another parameter that increased from 68.07% to 1.78% in these films. Li *et al*. (2017) developed chitosan films with lysozyme and rectorite, a type of layered silicate that can adsorb and stabilize other antimicrobial materials. According to their results, the tensile strength was reduced from 38.39 to 27.8 MPa concerning the chitosan films. The elongation percentage was also affected, obtaining a reduction from 12.44% to 8.42%, respectively. This could indicate a more significant interaction between the chitosan units and the lysozyme and rectorite.

The incorporation of essential oils can favor the reduction of water vapor permeability, as observed in studies done by Lamareerat *et al*. (2018), who had modifications in water vapor permeability in cassava starch and cinnamon essential oil films, reducing water permeability by increasing the essential oil content from 203.09 to 73.21 mm / m^2 day kPa. The mechanical properties of the films as the tensile strength decreased from 1.68 MPa to 0.31 MPa with the addition of essential oil. Opposite case with the elongation percentage, which increased from 92.38% to 281.06% compared to starch-only films. Similar results were reported by Bagde and Vigneshwaran (2019). They immobilized the bacteriocin produced by *Pediococcus acidilactici* strain on cellulose nanocrystals by adsorption method and were added to corn starch films. The water vapor permeability of the films was reduced from 1.9 g mm / Kpa m^2h^{-1} to 1.72 g mm / Kpa m^2h^{-1} concerning the control. The tensile strength increased from 3.1 MPa to 4.33 MPa, which indicated that there might have been increased binding between the polymer molecules and the immobilized bacteriocin.

Another study on the incorporation of essential oils is that of Simsek, Eke and Demir (2020), who studied the effect of essential oil on the physical, water vapor permeability, mechanical, optical and microstructural properties carboxymethyl cellulose films. Their results showed that as the essential oil content increased, the moisture content, solubility and water permeability of the films analyzed decreased. The mechanical properties and tensile strength increased, but the percentage of elongation decreased concerning the film control. Han, Yu and Wang (2018) reported an increase in thickness, oxygen and water vapor permeability, and elongation percentage of films.

Previous reviews have discussed the importance of components in the formulation of bio-based antimicrobial films, their interactions, production methods, and structures present, whether single or multi-layered. However, no emphasis has been placed on the importance and application of this type of packaging today. For this reason, in the following section, we will review their potential applications in the food area and the exponential growth in the research being carried out.

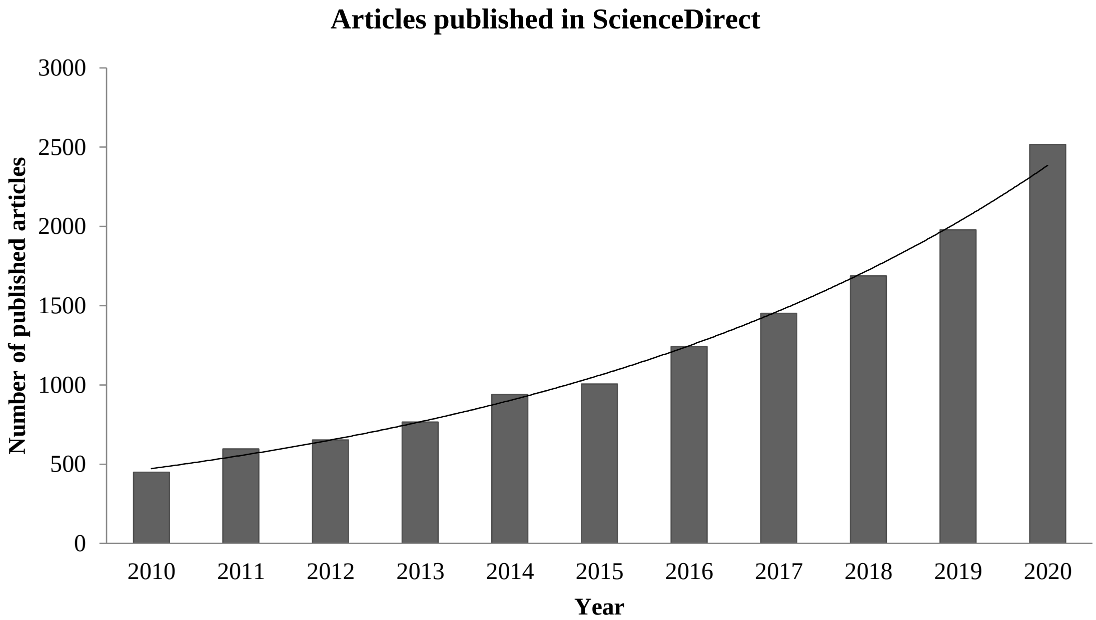
8. Importance of biodegradable antimicrobial packaging and its applications

The packaging industry's approach has been forced to make changes in packaging production, mainly to offer consumers safer products with a longer shelf life, in addition to caring for the environment by using materials from renewable sources such as bio-based biodegradable polymers. BBAs can include edible films or coatings made from proteins, lipids, polysaccharides, polylactic acid (PLA), polyhydroxybutyrate (PHB) and polyhydroxyalkanoate.

These qualities make BBAs relevant in the food industry. Their applications can range from natural products to ready-to-eat products. An example of these is the studies carried out in marine products where the BBAs inhibit the growth of pathogens such as *Salmonella, Listeria monocytogenes*, *Clostridium botulinum* and *Aeromonas hydrophila* or their application in bread to inhibit the growth of fungi such as *Penicillium commune, P. solitum, P. corylophilum, P. palitans* and several of the genus *Aspergillus,* which can produce unpleasant aromas in the product (Jideani & Vogt, 2015; Singh *et al.*, 2016).

Graph 1.1 shows the articles published using the keywords "Food packaging, Active packaging, antimicrobial packaging" as a search tool in the ScienceDirect database. An upward trend is observed in the articles published from 2010 to 2020 since the published number of articles was almost five times higher, which shows a constant development in this line of research, which seeks solutions for the different current problems of food safety and ecology.

Graph 1.1 Articles published in Sciencedirect on food packaging, active packaging and antimicrobial packaging



Among the articles published on this platform, we find antimicrobial packaging using active compounds such as silver nanoparticles, essential oils, bacteriocins and different types of polymeric matrices. They can range from simple unilamellar structures to structured films using nanocomposites or combinations of synthetic polymers to help reinforce the structure or change the final characteristics of the film. It can also be noted that the microorganisms most commonly used to test the inhibition of films are *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*, which are some of the most critical microorganisms causing ETAs.

According to studies conducted by Radha *et al*. (2015), the use of clove and cinnamon essential oils at a concentration of 4 % in films made from corn starch inhibited the growth of Gram-positive bacteria such as *Lactococcus lactis*, *Listeria monocytogenes*, *Leuconostoc mesenteroides* and Gram-negative bacteria such as *Pseudomonas fluorescens, Shewanella putrefaciens, Salmonella typhimurium* and *Escherichia coli*, using the disk diffusion method. Tests on meat samples showed a reduction in the growth of *Pseudomonas spp.* and *Enterobacteriaceae*.

Another study analyzing the inhibition of ETAs is those of Fatima *et al*. (2018), who used 2 % chitosan nanoparticles in PLA films. They obtained a reduction in the growth of *Listeria monocytogenes* of 67.09 % and 30.46 % for *Escherichia coli*. Ma *et al*. (2018) used PLA combined with PHB in a 3:1 ratio and 5 % cinnamaldehyde, obtaining films with the ability to maintain the growth of *Escherichia coli* and *Salmonella* below permissible levels in salmon for 17 days.

Souza *et al*. (2018) elaborated films with sodium chitosan-montmorillonite incorporating essential oil of ginger as an antimicrobial agent. Their results showed a reduction in the agent's activity when incorporated into a polymeric matrix, reducing the range of activity on different bacteria. The essential oil alone showed activity against bacteria such as *B. cereus*, *S. aureus* and *L. monocytogenes*. However, when incorporated into the films, these only showed activity against *B. cereus* and *S. enterica*. The application of antimicrobial agents in packaging provides a controlled release that increases the shelf life of different food products. However, their activity can be reduced.

Even with this problem, studies carried out in recent years have established an area of opportunity by meeting the objective of reducing the growth of different microorganisms in foods for a prolonged time. Table 1.3 shows some of the studies carried out in recent years on the preparation of packaging, the type of antimicrobial agent used, the preparation method, the polymeric matrix and the microorganism inhibited.

9. Future advances in antimicrobial active packaging

Throughout this study, an overview of the importance of antimicrobial packaging and bio-based polymers has been given. While antimicrobial agents are essential to conferring the activity of films, these can impact their structure. Another aspect that affects their structure is the method used for packaging formation, which can also affect the effectiveness of antimicrobials. For example, extrusion or thermo-pressing for packaging production offers advantages for mass production, but the shearing process or the use of high temperatures decreases the activity of the films. An innovative process has been proposed by Woraprayote *et al.* (2013), who used sawdust particles to assist the absorption of the active agent after the film is formed, resulting in the incorporated antimicrobial agents not being subjected to the production processes.

The processes of incorporating active agents are not the only area developed in the elaboration of packaging. The structural variations in films have been studied in the formation of multilamellar films and the controlled release processes by combining two release systems. Such is the case of Wu *et al*. (2015), who incorporated nanoliposomes with cinnamon essential oil in gelatin films. The antimicrobial activity of the films without the encapsulated agent was slightly higher in the first three days. However, the films with nanoliposomes showed better pathogen control after being stored for one month. This indicated better antimicrobial stability and prolonged activity. These results agree with those presented by Cui *et al*. in 2017, who encapsulated phages to control *Escherichia coli* in beef. Their results showed inhibition of free phages in the first 6 days, but samples with encapsulated phages showed activity for up to 15 days.

Other techniques that improve the physical properties of the films and achieve a reduction in the migration of the active agent are the implementation of ultraviolet (UV) rays, gamma rays, plasma, and the use of the electron beam. Within this group, plasma-treated films that cause a modification on the surface, where they break covalent bonds and form free radicals, stand out. Kolarova Raskova *et al*. (2018) evaluated the effect of this treatment on polyvinyl alcohol films containing nisin as an antimicrobial agent, showed that the treatment influenced the degree of nisin adhesion.

The use of irradiation is also an innovative technique to modify the release of active agents in packaging. This mechanism is based on its ability to induce crosslinking in the polymeric network, thus improving the physical properties of the films. Irradiation can bind functional groups on the surface, allowing the material to immobilize enzymes or other bioactive species. An advantage, also the incorporation of agents that promote the adsorption of compounds, is that it does not require temperature or hazardous chemicals for crosslinking. Lacroix *et al*. (2002) showed that gamma irradiation could induce crosslinking in calcium caseinate films, which allowed greater control of the release of enzymes and active compounds.

Undoubtedly, antimicrobial packaging of bio-based polymers is an extensive area of research, and new ways to design and produce them are being studied every year. In addition, new compositions and active agents are being used to enhance their characteristics further. The range of foods that can benefit from these developments is extensive; they can reduce foodborne diseases and the waste caused by microorganisms that deteriorate their composition, giving them undesirable characteristics. However, their implementation is not limited to the food area also can be implemented in the medical and cosmetic areas.

Table 1.3 Bio-based antimicrobial films tested on foods

Antimicrobial agent	Polymeric matrix	microorganism to inhibit	Food	Method of preparation	Type of packaging	Author
Silver nanoparticles	Glucomannan-chitosan	*Staphylococcus aureus* *Escherichia coli* *Candida albicans*	Slices of bread	Casting	Films	Nair, Alummoottil & moothandasserrry, 2016
Silver nanoparticles	Chitosan	*Botrytis cinerea*	strawberry	-	Coverage	Moussa, Teyel,Alsohim & Absallah, 2013
Silver nanoparticles	Chitosan	Mesophiles, psychrophiles, enterobacteria, molds and yeasts	Cut cantaloupe	-	Coverage	Ortiz-Duarte, Perez.cabrera, Arrés-Hermández & Martínez-Hernández, 2019
Ginger essential oil	Chitosan - sodium montmorillonite	*Bacillus cereus, Salmonella enterica*	Fresh poultry meat	Casting	Films	Souza et al., 2018
Cinnamaldehyde	PLA-PHB	*Escherichia coli, Salmonella*	Salmon	Casting	Films	Ma, Li & Wang, 2018
Chitosan nanoparticles	PLA	*Listeria monocytogenes and Escherichia coli*	Indian prawns	Casting	Films	Fathima, Panda, Ashraf,Varghese & Bindu, 2018
Nisin	cellulose	*Listeria monocytogenes*	Ham	Casting	Films	Yang, Liu, Wu & Lu, 2020
Carbachol, linalon, thymus	Methylcellulose - Hydroxypropyl methyl cellulose	*Aspergillus niger*	Cheddar cheese	Casting	Films	Kuorwel *et al.*, 2012
Cinnamon and clove essential oils	Corn starch	*Lactococcus lactis,Listeria monocytogenes, Leuconostoc mesenteroides, Pseudomonas fluorescens, Shewanella putrefaciens, Salmonella typhimurium, Escherichia coli.*	Raw meat	Casting	Films	Radha Krishnan *et al.*, 2015

10. Conclusion

Innovations in the area of bio-based antimicrobial packaging have demonstrated the efficiency of these technologies in preserving food. Not only do they help reduce losses caused by spoilage microorganisms, but they also reduce the incidence of ETAs and the use of plastics. For the formation of this type of packaging, food-grade biopolymers of natural origin can be used to obtain films or coatings. However, The production of bio-based antimicrobial packaging is limited because the interactions between their constituents, production method and type of structure have not yet been fully elucidated. In addition, these film production systems are costly and not yet regulated in food systems.

The increase in research studies responds to the demand of increasingly informed consumers for food preserved in biodegradable, innocuous packaging and even minimally processed products. The current challenges are obtaining bio-based films that compete with those derived from petroleum and production on an industrial scale. To this end, the industry must be interested in promoting research on this type of packaging system, highlighting the environmental importance and cost reduction by obtaining products with a longer shelf life.

The development of new technologies and processing methods would be another aspect that to be investigated since the traditional or proposed methods have disadvantages, such as the case of dry methods (thermo-pressing and extrusion) where the activity of antimicrobial agents is reduced, or the casting method, which is limited to laboratory use because films cannot be mass-produced.

The advantages offered by bio-based antimicrobial packaging in the food area are promising, especially in ready-to-eat or minimally processed foods, where shelf life is restricted to a few days. As packaging is designed specifically to the needs of each food, it helps to highlight its qualities and better preserve its sensory and nutritional properties.

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