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Minireview

Protein-based natural antibacterial materials and their applications in food preservation

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Summary

Plastics materials used for food packaging are recalcitrant, leading to a growing global environmental problem, which arouses the attention of environmental protection departments in many countries. Therefore, to meet the increasing demand for sustainable and environment-friendly consumer products, it is necessary for the food industry to develop natural antibacterial materials for food preservation. This review summarizes the common biodegradable natural antimicrobial agents and their applications in food preservation; as well as an overview of five commonly used biodegradable protein-based polymers, such as zein, soy protein isolate, gelatin and whey protein, with special emphasis on the advantages of protein-based biopolymers and their applications in food packaging industry.

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Introduction

Food packaging is usually used to preserve and protect food from oxidation and microbial decay, to prolong the shelf life of food (Tharanathan, 2003). Increased use of plastics packaging, which is derived from petroleum, has led to a serious environmental problem due to their total non-biodegradability (Atarés and Chiralt, 2016; Drzyzga and Prieto, 2019; Bhargava *et al*., 2020). Recently, people's awareness of environmental protection has been increasing dramatically, and environmental protection departments have paid more attention to the pollution of petrochemical-based plastics (Aires-Barros *et al*., 2019). Therefore, researchers are looking for new packaging materials and processes that are biodegradable, more friendly with the environment.

New biopolymers have been exploited to make edible and biodegradable films for eco-friendly food packaging (Azeredo, 2009). Biopolymers have many different classifications. Typically, they can be categorized based on their source of raw materials and manufacturing (chemical or conventional) processes as shown in Fig. 1 (Zubair and Ullah, 2020). Biopolymers or biodegradable polymers are groups of naturally accruing polymers such as proteins, lipids, carbohydrates, nucleic acid, etc. Commonly, biopolymers can be degraded into CO_2 and H_2O by microorganisms in a certain period of time (Sorrentino *et al*., 2007). However, the use of biopolymers for the development of biobased packaging materials on industrial level is limited due to their weak mechanical strength and barrier properties compared to plastic materials (Tang *et al*., 2012). For this reason, natural polymers were frequently blended with other synthetic polymers or chemically modified with the purpose of enabling their applications in food packaging (Zubair and Ullah, 2019; Cano *et al*., 2020).

The materials used in the preparation of biopolymers are either polysaccharides, proteins or lipids. However, due to the hydrophilicity of polysaccharides, polysaccharide-based films have poor barrier properties to water vapour. Lipid films are generally used as coatings because lipid-based films are relatively inelastic (Chen *et al*., 2019). Proteins have advantages, such as relative abundance, high

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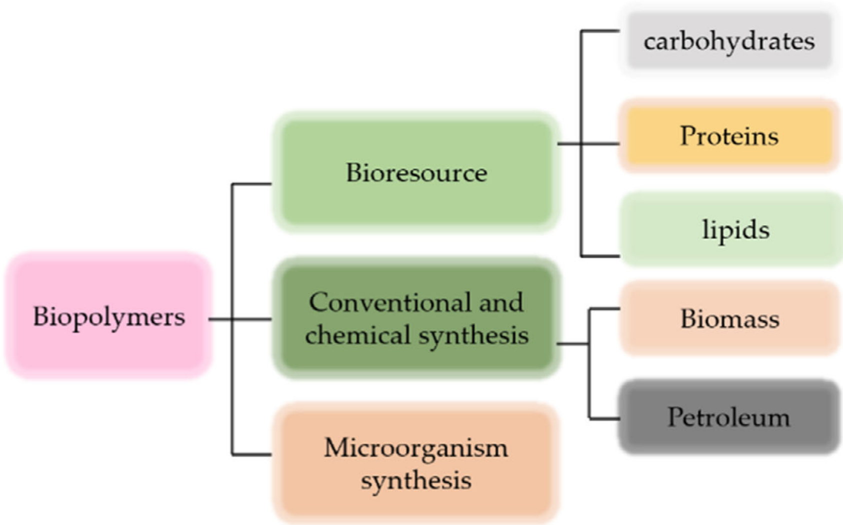

Fig. 1. Categories of biopolymers adapted from (Zubair and Ullah, 2019).

nutritional value and good film-forming ability when compared to polysaccharides and lipids. In addition, protein is considered one of the most important biopolymers which can be used as renewable raw material to develop environmentally friendly bioplastics, particularly for food packaging (Silva *et al*., 2015). Proteins are hetero-biopolymers with unique three-dimensional network structures as they are made up of different kinds of amino acids. The presence of various functional groups in the amino acids of protein chains offers excellent prospects such as exhibiting better gas barrier properties and mechanical properties (Calva-Estrada *et al*., 2019; Zubair and Ullah, 2019). Moreover, due to the amphiphilic properties of some protein-based polymers, it can carry active compounds such as various antimicrobial agents (Bahrami *et al*., 2020), which could preserve the quality of foods including nutrients.

Food spoilage is the process of contamination of foods causing several major negative effects on the sensory (flavour, colour and texture) properties of foods. In addition, the growth of pathogenic bacteria will also reduce the nutritional value of food, which deteriorates the quality of the product and makes it non-edible (Malhotra *et al*., 2015; Zuber and Brüssow, 2020). Therefore, protecting food from spoilage is considered to be very important at all stages of the food chain including production, storage and distribution. One of the main reasons for the deterioration of food quality is microbial growth (Biji *et al*., 2015; Malhotra *et al*., 2015). Recent food-borne microbial outbreaks (Campion *et al*., 2017; Han *et al*., 2018; Yong *et al*., 2018; Parlapani, 2020) are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety (Appendini and Hotchkiss, 2002). Although traditional food preservation methods, such as drying, heating, freezing, fermentation and pickling, can extend the shelf life of food, they are not perfect in inhibiting the growth of pathogenic microorganisms that may pose a problem to public health. Antimicrobial packaging is a novel development that incorporates antimicrobial agents into polymer films to suppress the activities of targeted microorganisms. Besides, antimicrobial packaging has been considered as the most promising methods which incorporated antimicrobial agents into food packaging system that helps in controlling the undesirable growth of a microorganism while extending the product's shelf life and safety (Gonçalves and Rocha, 2017).

However, antimicrobial packaging is still an extremely challenging technology because of its high cost and there are only a few commercialized products (Mirza Alizadeh *et al*., 2020) found in the market. Therefore, it is necessary to develop a practical, degradable and sustainable bio-antibacterial film that could reduce food losses and increase the shelf-life of food products. The protein-based film is one of the most promising ways to be used in designing active antimicrobial packaging applications (Said and Sarbon, 2019). In comparison to polysaccharides (such as starch and chitin) and lipids (such as waxes and paraffin), proteins possess superior film capacities because they have high mechanical and barrier properties (Zink *et al*., 2016). In addition, some proteins have weak antibacterial effects because of its hydrophobic structures, such as zein (Shukla and Cheryan, 2001).

This review briefly introduces the concept of antibacterial packaging, with an emphasis on the common natural antimicrobial agents and their applications in food storage. In addition, this paper summarizes the advantages of protein polymers in food packaging, including the most widely studied Zein, soy protein, gelatin and whey protein

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(WP)-based polymers. We invite readers to refer to Table 1, which provides an overview and promotes the development of antibacterial packaging in food-related fields. In summary, this paper describes the importance of natural antimicrobial agents, protein-based polymers and their applications in the food packaging industry.

Natural antimicrobial substances

Natural antibacterial agent refers to a class of substances with complex structure extracted from animals and plants in nature, or produced by the metabolism of microorganisms (Wright, 2019). According to their main sources, they can be divided into three categories: plant-derived natural antibacterial agents, animal-derived natural antibacterial agents and microbial-derived natural antibacterial agents. For many years, the food industry has been using preservatives such as sorbate, benzoate, nitrite and hydrogen peroxide to inhibit the growth of microorganisms that cause food spoilage. Those preservatives help prolong the life of food by disrupting the activities of pathogenic and spoilage microorganisms. However, they may have harmful effects on the sensory properties of certain foods (Falleh *et al.*, 2020). In order to ensure food quality, reduce health hazards and improve antibacterial efficiency, natural antibacterial agents, such as essential oils, lactoferrin and bacteriocins extracted from plants, animals or microorganisms, have gained wide attention of the food packaging industry due to their non-toxic, efficient and operable characteristics (Corbo *et al.*, 2009). This section mainly summarizes the common natural antibacterial agents of plant essential oils, plant extracts and antibacterial peptides. In addition, other natural antibacterial agents include propolis, glucose oxidase enzyme and probiotics.

Essential oils

Essential oils have been widely studied and applied as food antibacterial agents, mainly obtained from rosemary, ginger, oregano, sage and other plants by steam distillation or solvent extraction (Arfat *et al.*, 2014). Aldehydes, phenols and oxygen-containing terpenoids are the main antibacterial substances in essential oils. Their antibacterial mechanism is that these chemicals interfere with the structure of phospholipids of microbial feel and mitochondria, destroying the order of their structure and tissue, resulting in a massive loss of cell contents, important ions and molecules, and eventually cell death. At present, the main plant essential oils that have been developed for food antibacterial are: oregano essential oil, cinnamon essential oil, garlic essential oil, basil leaf essential oil, carvanol essential oil, lemon grass essential oil, eugenol essential oil, thymol essential oil, etc. (Tajkarimi *et al.*, 2010; Tu *et al.*, 2018; El-Saber Batiha *et al.*, 2021). They serve as antimicrobial, antioxidant compounds and are widely used in smart or bioactive packaging material to prevent the surface growth of microorganisms in foods (Tajkarimi *et al.*, 2010). Table 2 summarizes the antibacterial components and antibacterial activity of essential oils commonly used in recent years.

Botanical extracts and herbs

In recent years, spices and herbs are gaining attentions as potential sources of natural food preservatives due to the growing interest in the development of effective and safe natural food preservation. They have been used since ancient times to improve sensory characteristics of food, as preservatives, for their nutritional and healthy properties and also for their antimicrobial effect (Gyawali and Ibrahim, 2014). Plant extracts mainly include some alkaloids, glycosides, flavonoids, terpenoids, tannins and quinones, which have been proved to have broad-spectrum antibacterial properties and do not produce drug resistance (Ahmad Shiekh and Benjakul, 2020, Wang *et al.*, 2020; Efenberger-Szmechtyk et al., 2021b,2021a; Olatunde *et al.*, 2021).The antibacterial

Table 1. Features and applications of protein-based polymers.

Commonly used film-forming substrates	Features	Applications	References
Zein	Excellent biocompatibility and good film-forming ability	Food packaging materials, drug carrier, pork preservation	Mei *et al.* (2017), Kasaai (2018), Li *et al.* (2020)
Soy	Excellent film formation and oxygen barrier properties	Preservation of vegetables	Wu *et al.* (2017), Echeverría *et al.* (2018), Xu *et al.* (2019)
Gelatin	Excellent biocompatibility and film-forming properties	Preservation of meat products, vegetables、carbonated beverages	Clarke *et al.* (2017), Bermúdez-Oria *et al.* (2019), Amjadi *et al.* (2020), Umaraw *et al.* (2020), Mirzapour-Kouhdasht *et al.* (2021)
Whey	Excellent gelling and transparency, form good wall systems	Fresh cut turkey pieces, extend foodstuffs shelf-life	Brink *et al.* (2019), Talón *et al.* (2019)

Table 2. Natural antimicrobials from essential oils (EOs).

Examples	Application in food	Effect on microorganism	Bioactive molecule	References
Eugenol	Pork preservation	Higher against *E. coli* than against *L. monocytogenes* and *S. aureus*	The allylbenzene class of phenylpropanoids, phenolic acids, terpenes, aldehydes、terpenoids, ketones and acids;The compounds that exert strong antimicrobial activity have good structural configurations, chemical structure, especially the hydroxyl groups present in phenolic compounds	Cheng *et al.* (2019)
	Minced pork preservation	Inhibiting the increases in total bacterial counts (TBC)		Chen *et al.* (2021)
Oregano or clove essential oil	Cheese and pumpkin	The most significant antibacterial effects against *E. coli*		Requena *et al.* (2019)
Oregano essential oil	Active food packaging industry	Against three selected bacteria, E. coli O157:H7, *S. aureus* and *P. aeruginosa*		Liu *et al.* (2019)
Lemon essential oils	Prolonging shelf life of chilled pork	Effectively inhibit the growth of *E. coli*		Li *et al.* (2021)
Basil essential oil	Essential oil microcapsule-enriched mayonnaise	Significant antimicrobial activity against *E. coli* and *S. Typhimurium* in the mayonnaises		Ozdemir *et al.* (2021)
Cinnamon essential oil	Fish patty	Higher antimicrobial and antioxidant activities		Valizadeh *et al.* (2020)

mechanisms of plant extracts are mainly as follows: causing damage to the cell wall of microorganisms, entering into microbial cells to cause cytoplasmic condensation, destroying cell membrane and membrane proteins, etc. (Tian *et al.*, 2018; Efenberger-Szmechtyk et al., 2021b,2021a). Table 3 summarizes the plant extracts and herbs that have been reported so far, such as curcumin, tea polyphenols, pomegranate and grape seed extracts, and saponins.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are mostly composed of 12 ~ 60 amino acids with antibacterial activity, which

Table 3. Natural antimicrobials from botanical extracts and herbs.

Examples	Application in food	Effect on microorganism	Bioactive molecule	References
Tea polyphenol	Pork meat patties	Increase the effect of antimicrobial activity on pork meat	Phytochemical compounds such as polyphenols, terpenes, and aldehydes which exerts anti- oxidative effects and antibacterial	Qin *et al.* (2013)
	Extent the shelf-life of food products	Inhibition efficiency against the microorganisms of *S. aureus* and *E. coli.*		Feng *et al.* (2018)
	Fresh beef	Inhibiting the growth of microorganism		Gao *et al.* (2019)
Pomegranate peel	Develop bio-functional edible films	Exhibited better inhibition of *L. monocytogenes* and *E. coli*	A noteworthy source of phenolic compounds (ellagic acid, lignins, catechin, epicatechin, and ellagitannins)	Moghadam *et al.* (2020)
Fruit peels	Extend the shelf life of food products	Exhibited the best antimicrobial and anti-oxidant activities	Bioactive compounds such as sugars, minerals, fibres and phenols	Nur Hanani *et al.* (2018)
Gallic acid	As bioactive films	Had antimicrobial activity against *E. coli*	Phenolic compounds which exerts antimicrobial, anti-inflammatory and anti-cancer properties	Zarandona *et al.* (2020)
Aglycone and glycosidic saponins	Introduce new source of antibacterial compounds	Extracts were effective in antimicrobial activities	Saponins which exerts antibiotic and antifungal properties	Saboora *et al.* (2019)
Capsaicin	Fresh apple cubes	Endowed efficient antimicrobial activity against *E. coli*	Capsaicin has strong anti-microbial effects against Gram-negative bacteria, Gram-positive bacteria and fungi	Zhao *et al.* (2020)
Citric acid (CA)	As active food packaging	Reduced the *E. coli* growth	Bio-based polycarboxylic acid	Uranga *et al.* (2019)

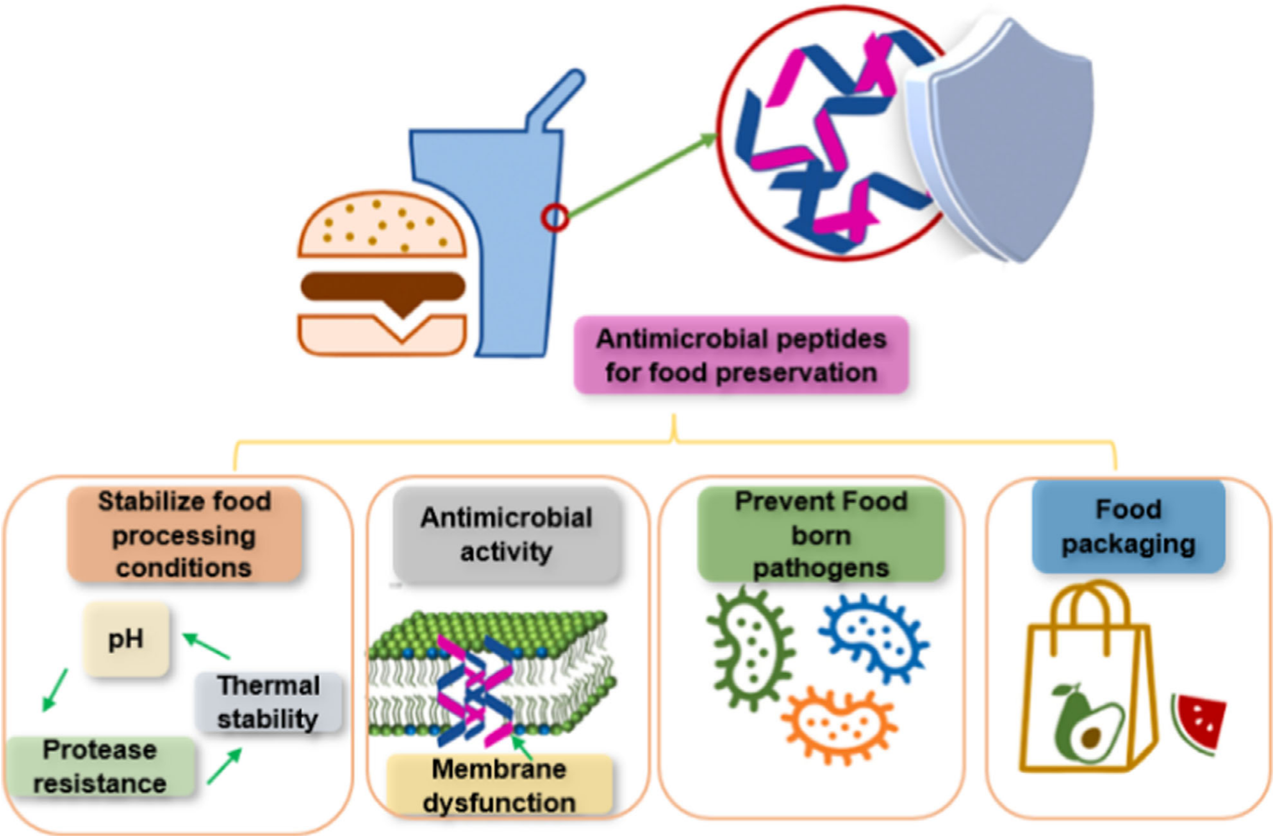

Fig. 2. Antimicrobial peptides for food preservation (Liu *et al.*, 2021).

widely exist in a variety of organisms and participate in the construction of host defence system (Liu *et al.*, 2021). These AMPs can have broad activity to directly kill yeasts, fungi, bacteria, viruses and even cancer cells. AMPs were first identified in the speckled frog, where they serve as a first line of defence against pathogenic microorganisms. Besides, also invertebrates, plants, bacteria and fungi, can produce AMPs as an innate response to infection (Zhang and Gallo, 2016; Mookherjee *et al.*, 2020). Cationic AMPs usually consists of 10–50 amino acid residues, and the total charge is positive. Additionally, AMPs exhibit a net positive charge and a high ratio of hydrophobic amino acids, allowing them to selectively bind to negatively charged bacterial membranes. This eventually results in perforation of the cell membranes and cellular content leaks and membrane potential collapse, what leads to cell death (Zhang and Gallo, 2016). This is different from the bactericidal mechanism of antibiotics, so it is not easy to make bacteria resistant. Furthermore, the AMPs are not easy to bind to mammalian cell membranes, which is very harmful. Antimicrobial peptides generally have broad-spectrum antibacterial properties, non-toxic side effects, good stability and not easy to produce drug resistance. Some AMPs also have the functions of antioxidation and scavenging free radicals. Therefore, AMPs have huge application prospects in medicine, food and other industries. Antimicrobial peptides mainly prevent the oxidative deterioration of food components by inhibiting the proliferation of spoilage microorganisms, so as to achieve the purpose of delaying food spoilage. Based on the sustained release of bioactive compounds such as AMPs, it can maintain the quality of food and extend its shelf life (Liu *et al.*, 2021). Figure 2 describes the function of AMPs in food preservation (Liu *et al.*, 2021). Table 4 summarizes the recent literature on AMPs in food storage.

Protein-based antibacterial materials and application in food

Zein-based polymers

Zein, the major storage protein in corn endosperm, is a Generally Recognized As Safe (GRAS) food-grade ingredient (Weissmueller *et al.*, 2016). Like other proteins, zein-based materials are also potentially biodegradable and environment friendly. Based on the structure, it consists of four types (α, β, γ and δ) having

Table 4. Various AMPs and their function in active packaging.

Form of use	Antimicrobial peptide	Application in food	Effect on microorganism	References
Liposomes/ nanoliposomes	Peptide hydrolysate	An innovative edible wrapping material with antimicrobial activity	Effective against *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*	Alemán *et al.* (2016)
Film composite	Lactoferrin	Antimicrobial edible film composite	Including against Gram-positive or Gram-negative bacteria, yeasts, viruses, etc	Dinika *et al.* (2020)
Lysozyme-containing polyelectrolyte complexes.	Lysozyme	The preservation of real food products	Sustained antimicrobial activity	Ozer *et al.* (2016)
gliadin films cross-linked with cinnamaldehyde		Develop sustained active compound release systems for many applications	Exerted the highest antimicrobial activity against the microorganism evaluated	Fajardo *et al.* (2014)
The peptides emulsions	Peptide fractions obtained from barred mackerel gelatin	As an ingredient for the fortification of carbonated beverages	Indicating the potency of this fraction against *E. coli* cells	Mirzapour-Kouhdasht *et al.* (2021)
Microparticles	Nisin	Biopolymers as delivery vehicles for antimicrobial agents	Show antimicrobial activity against Gram-positive bacteria	Chandrasekar *et al.* (2017)
Development of a sensitive biosensor		Detect *Salmonella* in milk	Attacking bacteria and destroying the cell membranes	Malvano *et al.* (2020)
Nanoparticles		Increases the shelf-life of the fresh tomato juice	Confirmed antimicrobial activity against Gram-positive bacteria, such as *Listeria monocytogenes*, *Bacillus subtilis* and *Staphylococcus aureus*	Luo *et al.* (2019)

different peptide chains, molecular sizes and solubilities. The most abundant protein in commercial zeins is the alpha type comprising 70–85% of the whole zein which is soluble in ethanol (Shukla and Cheryan, 2001). Zein has many hydrophobic amino acids that are the main reason for its unique solubility and hydrophobicity. Thus, some studies have shown commercial zeins are soluble in aqueous ethanol, aqueous acetone and aqueous alkaline solutions with pH≥ 11.5, and poorly soluble in water alone (Shukla and Cheryan, 2001). Besides, zein, a commercially available agricultural product, is soluble in hydroalcoholic mixtures from which it can be easily converted into transparent films with attractive properties due to the high content of non-polar amino acids, such as good moisture barrier and excellent oxygen barrier (Kashiri *et al.*, 2016).

Therefore, zein is an ideal alternative candidate for the synthetic polymers for food packaging due to its biodegradability, sustainability, good film-forming ability and adhesive/cohesive properties (Zhang *et al.*, 2018; Shi *et al.*, 2019). In addition, zein proteins are 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 (Shukla and Cheryan, 2001; Shi *et al.*, 2021). However, the film is brittle and tough. Thus, fatty acids or cross-linking agents are used to improve the water vapour barrier characteristics of zein films and coatings. Many antimicrobial agents have been incorporated into zein-based polymers such as metal ions, essential oils, natural extracts, polymers, organic acids and bacteriocins which resulted in great inhibition toward the growth of microorganism and pathogens (Cristina *et al.*, 2015).

For example, Aytac *et al.* (2017) developed Thymol (THY)/c-Cyclodextrin(c-CD) inclusion complex (IC) encapsulated electrospun zein nanofibrous webs (zein-THY/c-CD-IC-NF) as a food packaging material (Fig. 3). In their work, the results showed that Zein/c-CD-IC-NF (2:1) had the best inhibitory effect on bacterial growth in meat products. Besides, Wang *et al.* (2019) have reported an innovative strategy for constructing nanofibril films by using konjac glucomannan (KGM), zein and loading curcumin (Cur) which indicated an excellent antibacterial activity against food-borne pathogens. In this study, they confirmed the interactions of hydrogen bonds between KGM and zein and the addition of zein caused an increase of thermal properties and hydrophobicity. Besides, they showed that the KGM/Zein/Cur composite nanofibril film exhibited excellent antibacterial effects on *Escherichia coli* and *Staphylococcus aureus* with a large halo inhibition zone of 12–20 mm (Wang *et al.*, 2019). Besides, some other studies reported that zein-based composite film loaded with thymol (Li *et al.*, 2020a,2020b), Lauroyl-L-arginine ethyl ester monohydrochloride (LAE) (Kashiri *et al*., 2019) and other antibacterial agents inhibited the growth of microbes. In addition, the combination with essential oil antibacterial agents, there are also many studies on the combination of zein and plant extracts. For instance, li and his colleagues (Li *et al*., 2020a,2020b) developed antimicrobial protein films by using resveratrol (R) and gelatin/zein (GA/ZN). The fibre mats could extend the pork meat shelf life, showing the good application prospect in food preservation. Furthermore, in order to enhance the antimicrobial activity of polymer-based food packaging materials, in Aytac's recent work, she developed a cocktail of nature-derived antimicrobials including thyme oil, citric acid and nisin (Fig. 4). These antimicrobial fibres effectively reduced *E. coli* and *Listeria innocua* populations and they have the potential to be developed into a sustainable food packaging material (Aytac *et al*., 2020). Thus, based on the above summary, it can be concluded that zein-based film does result in good application prospect with incorporation of antimicrobial agents.

Soy protein isolate polymers

Soy protein is one of the increasingly important food proteins in human diets and has been recognized to be nutritional, functional and even health-benefiting (Tang, 2019). Soy protein isolate (SPI), a protein with reproducible resource, good biocompatibility, biodegradability and processability, is the most abundant plant protein in nature among varieties of biopolymers and it has significant potential in the food industry, agriculture, bioscience and biotechnology (Karen and Ramille, 2011; Song *et al*., 2011; Nuno *et al*., 2014). Soy protein isolate is usually a by-product of the soybean oil industry. Like other globular proteins, SPI shows good film-forming properties and is therefore suitable for edible films and coatings (Zhang *et al*., 2010). Moreover, SPI-based films are clearer, smoother and more flexible compared to other plant protein-based films and they have impressive gas barrier properties compared with those films prepared from lipids and polysaccharides (Guilbert, 1986). However, SPI films do not show satisfactory mechanical properties or water vapour barrier properties in practical applications due to the inherent hydrophilicity and the strong molecular interaction of natural protein, and these properties become poorer under highly humid conditions (Song *et al*., 2011). To avoid brittleness, a plasticizer must be added for film formation and glycerol is the plasticizer most often used (Zhang *et al*., 2010).

In all, soy protein in addition to its nutritional and beneficial health effects, also exhibits a number of good functionalities such as the high tendency to aggregate and gelation, good film-forming properties. And they have been widely used as a kind of important antibacterial SPI-based film for food packaging.

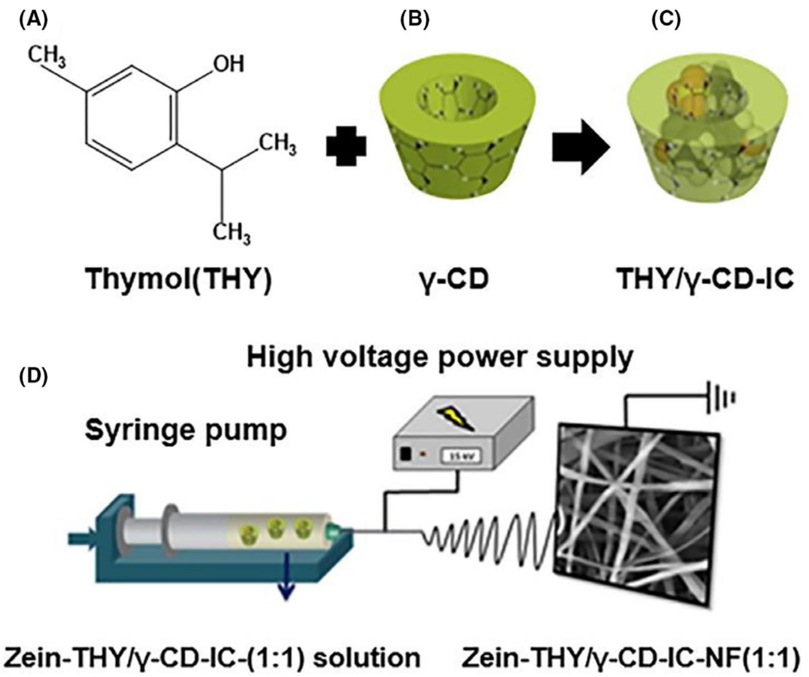

Fig. 3. The chemical structure of (A) THY; (B) schematic representation of c-CD, (C)THY/c-CD-IC formation and (D) electrospinning of nanofibers from zein-THY/c-CD-IC (1:1) solution (Aytac *et al*., 2017).

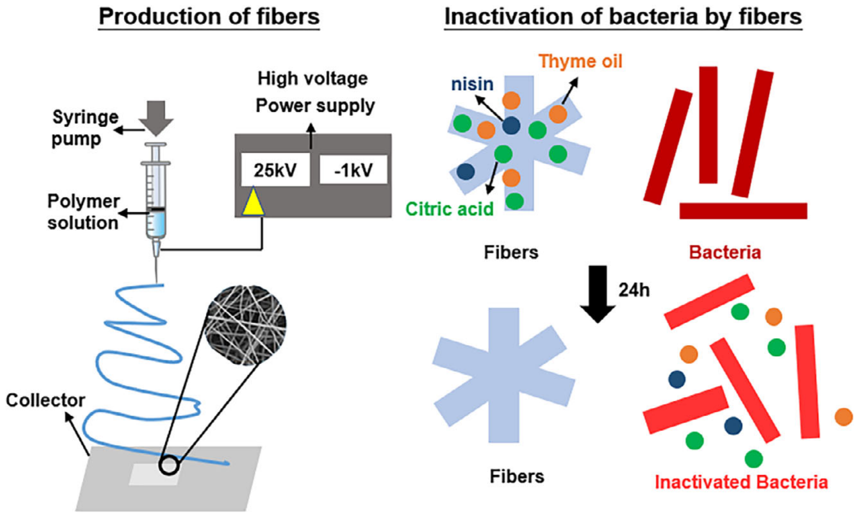

Fig. 4. A cocktail of nature-derived antimicrobials was developed and included thyme oil, citric acid and nisin (Aytac *et al*., 2020).

Recently, Wu *et al*. (2021) developed antimicrobial protein films by using diatomite/thymol (D/T) complex and SPI. Thus, the S/D/T film showed better mechanical and water vapour barrier properties compared to the S/D film. More importantly, a delayed and sustained release of thymol from the film was observed thus enhancing antibacterial effects of S/D/T film on *E. coli* (Wu *et al*., 2021) (Fig. 5). In a study conducted by Sivarooban, *et al*. (2008) they evaluated the physical and antimicrobial properties of SPI films containing nisin (10 000 IU g^{-1}), grape seed extract (GSE 1% w/w), ethylenediaminetetraacetic acid (EDTA 0.16% w/w) and their combinations. The soybean protein edible membrane effectively inhibited the growth of *L. monocytogenes*, *E. coli* and *Salmonella typhimurium*. Furthermore, in a recent study by Pan *et al*. (2018), they demonstrated that soy protein and egg white protein can preserve nisin activity from trypsin enzymatic hydrolysis solving the shortcoming of nisin's sensitivity to protease in the application. Thus, nisin will be more widely used during meat processing.

Gelatin protein-based polymers

Gelatin, one of the renewable and sustainable proteins, is obtained by hydrolysing the collagen contained in the bones and skin of animals. Gelatin is composed of a unique sequence of amino acids and the characteristic features of gelatin are the high content of the amino acids glycine, proline and hydroxyproline. Gelatin also has a mixture of single and double unfolded chains of a hydrophilic character (Wittaya, 2012). Films formed by using gelatin sources as a primary biopolymer are more desirable to produce as they are available and low cost (Clarke *et al*., 2017). Besides, 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 (Said and Sarbon, 2019).

For instance, Shankar *et al*. (2015) reported that Gelatin-based zinc oxide (ZnO NPs) nanocomposite films showed profound antibacterial activity against both Gram-negative and Gram-positive foodborne pathogenic bacteria. Thus, this research presented that the nanocomposite film could be applied for food preservation by controlling foodborne pathogens. Zhao *et al*. (2020) prepared antibacterial gelatin/chitosan (CH) film containing capsaicin loaded Fe^{III} doped hollow metal-organic frameworks (Fe^{III}-HMOF-5) (Fig. 6). In this study, capsaicin, an effective extract of red chili, is thought to be a favourable antibacterial candidate in food packaging. Besides, Cap-Fe^{3+}-HMOF-5 endowed efficient antimicrobial activity against *E. coli* to Gel/Chi composite films by practical application on fresh apple cubes. Wu *et al*. (2015) prepared gelatin films containing cinnamon essential oil (CEO) nanoliposomes to enhance the antimicrobial stability by thin film ultrasonic dispersion method. Their results indicated that the incorporation of CEO nanoliposomes as a natural antibacterial agent possessed sustained release effect, which had potential for using the developed film as an active packaging. And in this study, the results suggested that the prepared CEO nanoliposomes improved antimicrobial stability and prolonged the antimicrobial time (Wu *et al*., 2015). And a sturgeon skin gelatine film combined with esculin and ferric citrate was developed by Tian *et al*. (Tian *et al*., 2020) as an edible food packaging material to

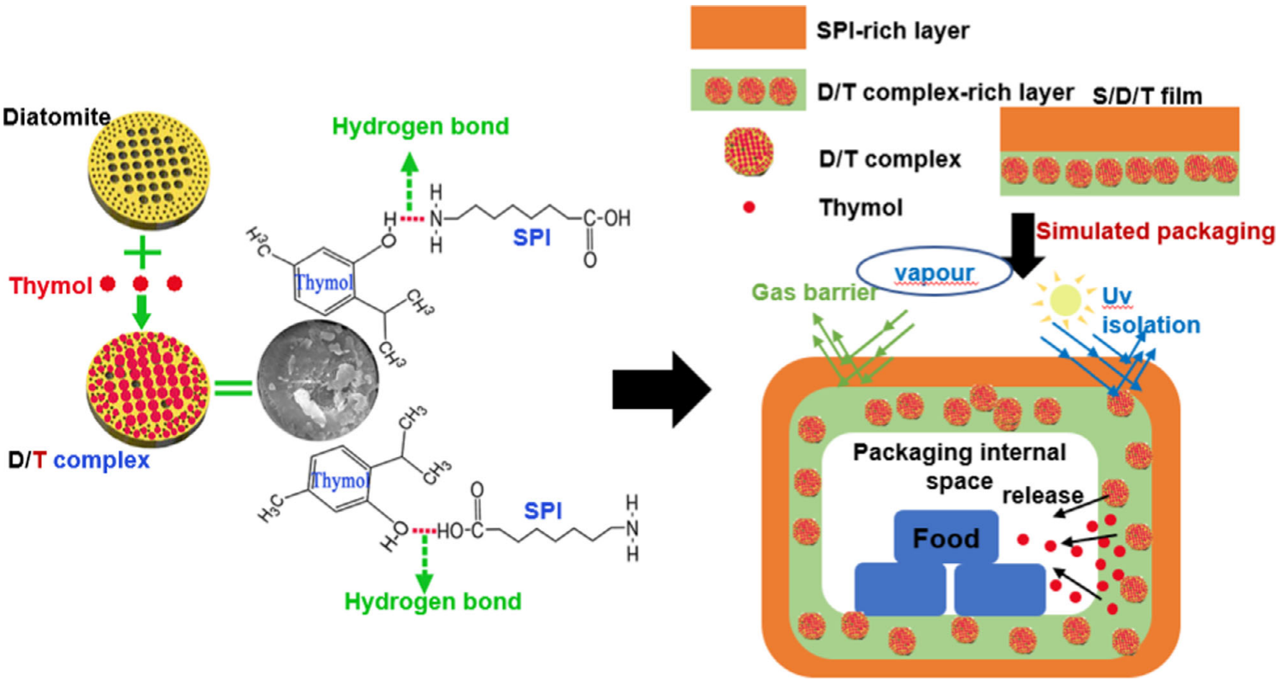

Fig. 5. Schematic diagram for proposed active food packaging systems using S/D/T film (Wu *et al.*, 2021).

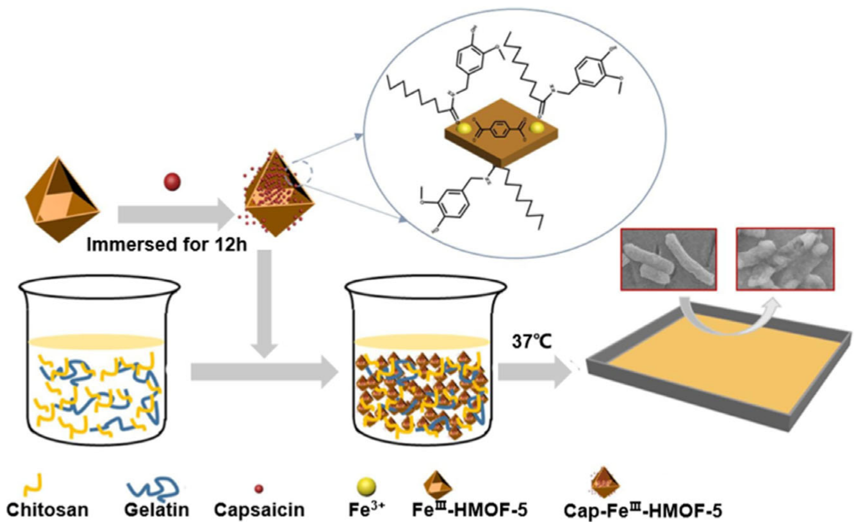

Fig. 6. Schematic diagram of the preparation of Cap-Fe^{III}-HMOF-5 modified Gel/chi film (Zhao *et al.*, 2020).

prevent *Enterococcus faecalis* (*E. faecalis*) contamination. Besides, in a study conducted by Sáez-Orviz *et al.* (2020), the gelatine films with PLA-thymol nanoparticles showed high transparency, a homogeneous microstructure and antimicrobial properties. In addition to the combination of the above gelatin and antibacterial agents, Eun and other researchers used fish by-products to produce gelatin peptides with antioxidant and antibacterial functions, and applied them to carbonated beverages. This research showed that the antibacterial activity of the lowermost molecular weight fraction (F < 3 kDa) of fish waste protein hydrolysates against both Gram-positive and negative bacteria was significantly higher than other fractions tested. Based on the above, it can be concluded that gelatin-based antibacterial film has been widely studied while showing great potential as a medium to release or emit active antimicrobial agents against the growth of pathogenic bacteria.

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Whey protein-based polymers

Whey proteins, which are a by-product of cheese and casein manufacturing, are the soluble constituent of milk and represent about 20% of the proteins in cow milk (Coltelli *et al*., 2015). The most abundant proteins in whey are β-lactoglobulin followed by α-lactalbumin with the most sulfhydryl groups and hydrophobic residues in the interior of the molecule. The formation of WP films usually consists of heat denaturation of WPs in aqueous solutions. Studies on the utilization of WPs mainly focus on their chemical, biochemical and bioactive properties for the development of food, biotechnology, medicine and biodegradable materials (Qi and Onwulata, 2011). Whey proteins are considered appealing because they are able to build structures of transparent, odourless and flexible films (Lacroix and Cooksey, 2005). In addition, WP-based films are one well-studied biodegradable film due to their several desirable properties including good appearance, high elasticity and moderate oxygen barrier (Çağrı Mehmetoğlu *et al*., 2020). However, the usage of WP films and coatings in the food industry has some limits due to their hydrophilic properties, poor tensile strength and low water vapour barrier properties. 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 (Said and Sarbon, 2019). An example is the combination of proteins and lipids to form biodegradable edible films and coatings, whereby the low water vapour resistance of protein films is compensated for by the wiper-repelling properties of the incorporated lipids (Kashiri *et al*., 2017). Besides, WP films have proven to be good vehicles for antimicrobial agents such as malic acid, nisin and natamycin (Brink *et al*., 2019).

In Talón's research, eugenol was coated with WP, soy lecithin, maltodextrin, oleic acid and CH by spray drying to obtain antioxidant and antibacterial powder for food (Talón *et al*., 2019). Furthermore, a study showed that in WP isolate biodegradable films, citric acid is added to increase the antimicrobial, plasticizing and dispersing effect of montmorillonite in nanocomposites and to improve the mechanical properties and water vapour permeability (Azevedo *et al*., 2015). Besides, a study reported by Brink et al. (2019) that edible films were made from a mixture of WPs and CH, supplemented with cranberry or quince juice, and then applied on fresh-cut turkey pieces. In this study, the researchers hypothesized that such films could combine the antimicrobial activity of CH and that of cranberry or quince juice due to the presence of organic acids. In their initial works, they demonstrated the antifungal activity of edible films made from cranberry and quince juice and applied to fresh cut apples (Simonaitiene *et al*., 2015). In addition to the above research, Altinkaya *et al*. developed a novel controlled release system using pH-responsive polyacrylic acid (PAA)/lysozyme (LYS) complexes incorporated within a hydrophilic whey protein isolate (WPI) film matrix. And the results showed that complexation of lysozyme with weak polyelectrolytes can achieve a long-lasting antimicrobial effect and that films prepared have great potential in food packaging (Ozer *et al*., 2016) (Fig. 7). Whey protein-based antibacterial film also has excellent results toward the growth of microorganisms as

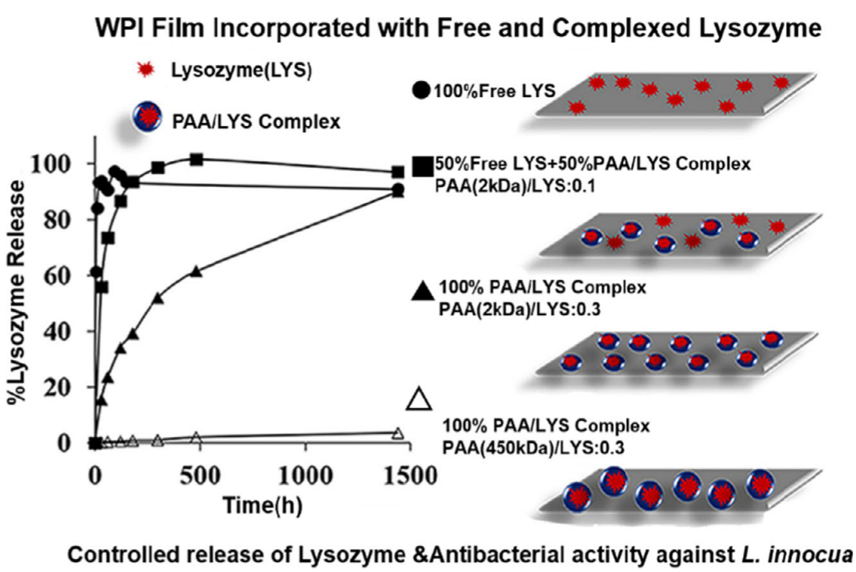

Fig. 7. A hydrophilic whey protein isolate (WPI) film matrix for active food packaging applications was developed and included polyacrylic acid (PAA)/lysozyme (LYS) complexes (Ozer *et al*., 2016).

it provides good polymeric matrices for the antimicrobial agents to emit the active compounds into the packaging system (Ozer *et al*., 2016).

In summary, the protein-based antibacterial film has gained great interest due to its potential application as 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, functionality, stability and shelf life of food products (Said and Sarbon, 2019).

The real-time quality evaluation of perishable products like fish, meat, fruits and vegetables, milk, and dairy product, is in great demand that is fulfilled by intelligent packaging systems (Qin *et al*., 2020). To extend the food shelf life and quality, a number natural polymers could be used in bio-packaging, such as CH, starch, pectin, alginate, proteins etc. (Mohamed *et al*., 2020). In order to ensure the safety and quality of the meats and products, the protein-based packaging is widely used and some packaging methods were developed (Emiroğlu *et al*., 2010; Alparslan *et al*., 2014; Coskun *et al*., 2014; Nagarajan *et al*., 2015). The most appropriate protein source, additives and methods will be chosen according to the nature and requirements of the food, the nature and degree of the protection required, the shelf life and so on (Chen *et al*., 2019). Meanwhile, protein-based packaging could be used together with nonedible film as multilayer food packaging materials where it can be designed as internal layers that have direct contact with food materials (Wittaya, 2012).

Conclusion and outlook

In recent years, with increasing environmental and sustainable concerns about petroleum-based polymer materials used in food package, the development of biodegradable bio-based materials is attracting more and more attention. Therefore, for the research and use of biodegradable natural antibacterial materials, we not only pay attention to their applications in food preservation but also should carry out the research and exploration of its raw materials and preparation methods to prolong the shelf life of food. Proteins, with unique three-dimensional network structures, offer excellent advantages such as exhibiting better gas, aroma-barrier properties and acceptable mechanical properties compared to carbohydrates and lipids. Antimicrobial agents are also used as additives in films to help preserve for a longer period. However, protein-based antibacterial films with antibacterial agents still show poor both water vapour resistance and mechanical strength in comparison with synthetic polymers and this limits their application in food packaging. Thus, various modification strategies, including bulk and surface modifications, were utilized to improve mechanical properties and water resistance of protein-based films. In addition, with the continuous progress of science and technology, there are some problems in some packaging systems using natural antimicrobial agents, such as the disunity of the evaluation and detection methods of the antibacterial effect of natural antimicrobial agents, and the lack of understanding of the antibacterial mechanism of natural antimicrobial agents. Improper control of the addition amount of antimicrobials makes them spread and migrate to food, which causes some hidden dangers to food safety. In addition, plant essential oils may affect the flavour of packaged food and the food safety problems caused by the use of AMPs. Therefore, for the research and use of natural antimicrobial agents, we should not only pay attention to its application in food anticorrosion; in the future, we should also carry out multi-disciplinary advanced technologies such as microbiology, migration kinetics, and active packaging materials to realize the best use of natural antimicrobial agents in the field of food packaging, so as to reduce food pollution and food safety problems. More in-depth exploration of natural antimicrobial agents has a certain strategic significance, and its application in the field of food fresh-keeping packaging has a broader prospect.

Recently, the application of nanocomposites promises to expand the use of edible and biodegradable films. It will help to reduce the packaging waste associated with processed foods and will support the preservation of fresh foods, extending their shelf life. These films have an upcoming potential in the food industry. However, their use is not a substitute for good sanitation practices, but it enhances the safety of food as an additional hurdle to the growth of pathogenic microorganisms.

Based on the above, the results show that the protein-based antibacterial films developed have the potential to replace the traditional plastic packaging. Thus, protein-based antibacterial films can be optimized and commercialized. It is encouraging that with the development of smart packaging, active packaging, antibacterial packaging and nanotechnology, protein-based antibacterial films will develop better in the future.

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Conflict of interest

None declared.

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