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Impact of adding prebiotics and probiotics on the characteristics of edible films and coatings- a review

S. Sáez-Orviz, M. Rendueles *, M. Díaz

Department of Chemical and Environmental Engineering, University of Oviedo, C/Julián Clavería 8, 33006 Oviedo, Spain

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ABSTRACT

Nowadays, conventional packaging materials made using non-renewable sources are being replaced by more sustainable alternatives such as natural biopolymers (proteins, polysaccharides, and lipids). Within edible packaging, one can differentiate between edible films or coatings. This packaging can be additivated with bioactive compounds to develop functional food packaging, capable of improving the consumer's state of health. Among the bioactive compounds that can be added are probiotics and prebiotics. This review novelty highlighted recent research on edible films and coatings additivated with probiotics and prebiotics, the interactions between them and the matrix and the changes in their physic, chemical and mechanical properties. When bioactive compounds are added, critical factors must be considered when selecting the most suitable production processes. Particularly, as probiotics are living microorganisms, they are more sensitive to certain factors, such as pH or temperature, while prebiotic compounds are less problematic. The interactions that occur inside the matrix can be divided into two main groups: covalent bonding ($-NH_2$, -NHR, –OH, $-CO_2H$, etc) and non-covalent interactions (van der Waals forces, hydrogen bonding, hydrophobic and electrostatic interactions). When probiotics and prebiotics are added, covalent and non-covalent interactions are modified. The physical and mechanical properties of films and coatings depend directly on the interactions that take place between the biopolymers that form their matrix. Greater knowledge about the influence of these compounds on the interactions that occur inside the matrix will allow better control of these properties and better understanding of the behaviour of edible packaging additivated with probiotics and prebiotics.

1. Introduction

Packaging is an ancient technology that has been used since ancient times to prevent or delay the deterioration of food products (Ribeiro, Estevinho, & Rocha, 2021). Packaging is of fundamental importance during the storage and transport of food products, as there are many physical, chemical and microbiological challenges that can affect the stability of foodstuffs and compromise their quality (Jeevahan et al. 2020) (Ribeiro et al., 2021). The materials most often used in food packaging are paper or paperboard, metal, or glass, but plastic is the preferred type of packaging. Plastic is a cheap, lightweight and very versatile material (Jeevahan et al. 2020), but nevertheless, has serious environmental drawbacks, as it is non-biodegradable and comes from a non-renewable source (Parreidt, Müller, & Schmid, 2018). During the manufacture of this type of packaging, a variety of substances (such as carbon monoxide, hydrochloric acid, amines, benzenes, etc. (Amin et al., 2021)) are emitted. Besides the environmental issues these emissions raise, they also cause health problems (Mangaraj, Yadav, Bal, Dash, & Mahanti, 2019) and it is for these reasons that nowadays conventional packaging materials derived from petroleum and other non-renewable sources are being replaced by more sustainable alternatives.

Consumers have begun to demand minimally processed foods and the use of edible packaging can help to extend the shelf life and improve the quality of such food products. In this context, the use of biopolymers of natural and renewable origin is drawing attention (Parreidt et al., 2018) and proteins, polysaccharides, and lipids have been used as

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Abbreviations: CLA, Conjugated linoleic acid; EFSA, European Food Safety Authority; EU, European Union; FDA, Food and drug administration; FOS, Fructo-oligosaccharides; FTIR, Fourier transform infrared; GIT, Gastrointestinal tract; GOS, Galacto-oligosaccharides; GRAS, Generally recognized as safe; IMO, Isomalto-oligosaccharides; LAB, Lactic acid bacteria; PUFAs, Polyunsaturated fatty acids; QPS, Qualified presumption of safety; SEM, Scanning electron microscopy; SOS, Soya-oligosaccharides; WVP, Water vapour permeability; WVTR, Water vapour transmission rate; XOS, xylo-oligosaccharides.

* Corresponding author.
E-mail address: mrenduel@uniovi.es (M. Rendueles).

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primary material for the development of edible biodegradable packaging. Edible biodegradable packaging materials have been categorized as films and coatings (Amin et al., 2021), the main difference being the manner of application in the food products (Parreidt et al., 2018). Edible films and coatings are very versatile and have the potential to include bioactive compounds in their matrix, which is one way in which value can be added to the packaging market, since food products can be transformed into "functional" foodstuffs. Functional food can be defined as "food that beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease" (Ashwell, 2002). Among the active compounds that can be added are probiotics (Espitia, Batista, Azeredo, & Otoni, 2016) and prebiotics (Paulo, Baú, Ida, & Shirai, 2021).

Probiotics are living microorganisms with beneficial effects on humans and animals (Food and Agriculture Organization (FAO) of the United Nations, 2002) (Espitia et al., 2016). The most common probiotics employed in food products belong to the genera *Lactobacillus* and *Bifidobacterium* (Espitia et al., 2016). Probiotic bacteria have been used since ancient times to ferment food products such as yogurt, cheese, dry cured meat, and fermented vegetables, among others. (Hellebois, Tsevdou, & Soukoulis, 2020). For probiotics to be effective, on reaching the gastrointestinal tract (GIT) they must be alive and present in sufficiently large amounts (Zendeboodi, Khorshidian, Mortazavian, & da Cruz, 2020). Their inclusion in edible films and coatings is a way of increasing their viability and survival during food production processes, since probiotics are living microorganisms and there are several physical and chemical factors that can threaten their survival (Mbye et al., 2020).

As for prebiotics, they are non-digestible ingredients that beneficially affect consumers by stimulating the growth of certain bacteria in the GIT. They comprise a wide range of compounds of both animal and vegetal origin which have been commonly used directly in products such as beverages, bakery products or dairy products (Paulo et al., 2021). However, during production processes, their functionality can be compromised by very high or very low temperatures, changes in pH and Maillard reactions (Neri-Numa & Pastore, 2020). Thus, edible films and coatings have the capacity to protect them. In addition, several studies have observed that when probiotic bacteria are combined with prebiotic compounds, their viability is improved, both during the food production process and during storage and even in *in vitro* digestion tests (Espitia et al., 2016). The combination of probiotics and prebiotics in the same food product gives rise to what is called a synbiotic foodstuffs (Hellebois et al., 2020).

When selecting the production methods for edible films and coatings, it is necessary to consider the parameters that can affect the viability of probiotics (Mbye et al., 2020). Besides, the addition of prebiotics and probiotics leads to changes in the molecular interactions between the components of the biopolymeric matrix and these will produce structural changes, affecting the physical and mechanical properties of the edible films and coatings being developed.

Bearing in mind the objectives, ideas and requirements mentioned above, this review aims to summarize current understanding of the natural biopolymers that can be used to develop edible films and coatings, as well as the probiotics and prebiotics commonly used in this type of packaging. In addition, the advantages and disadvantages of the different production methods and the key factors to be considered when adding probiotics and prebiotics will be analysed. This review also highlights for the first time the molecular interactions that take place inside the packaging matrix, how they are affected by the presence of probiotics and prebiotics and the changes in physical and mechanical properties that occur in edible films and coatings. Finally, the regulation and commercialization of this type of packaging will be discussed.

2. Use of biopolymers as matrix in edible packaging

Nowadays, the use of conventional petroleum-based and synthetic plastics is being replaced, at least partially, by environmentally friendly alternatives (Rojas-Lema et al., 2021) (Liminana, Garcia-Sanoguera, Quiles-Carrillo, Balart, & Montanes, 2018) and it is in this context that edible packaging using biodegradable polymers as the matrix has emerged. The main biopolymers used in the development of edible packaging are proteins, polysaccharides and lipids, and these compounds may be obtained from different sources, both animal and vegetable. Thus, these biopolymers may be obtained from the by-products of other industries, such as agro-industrial and marine wastes, which can make it possible to reuse and take advantage of them as part of a circular economy (Ribeiro et al., 2021) (Chiralt, Menzel, Hernandez-García, Collazo, & Gonzalez-Martinez, 2020).

In the case of proteins, the most common used in the preparation of edible packaging are whey protein, casein, corn zein, gelatine, soy protein and wheat gluten (Amin et al., 2021) (Zoghi, Khosravi-Darani, & Mohammadi, 2020). Proteins have hydrophobic and hydrophilic parts, which allows them to be used in combination with different bioactive compounds (Calva-Estrada, Jiménez-Fernández, & Lugo-Cervantes, 2019) (Hassan, Chatha, Hussain, Zia, & Akhtar, 2018). The final characteristics of the films and coatings developed depend to a large extent on the type of protein they are made of, since properties such as molecular weight, conformation, charge, flexibility, and thermal stability will be different (Koshy, Mary, Thomas, & Pothan, 2015). Packaging made with protein as a matrix usually has very good mechanical and barrier properties (Calva-Estrada et al., 2019) (Ribeiro et al., 2021). However, these films are usually very soluble in water, which makes their use in the food industry very difficult, since most foods contain a high percentage of water (Gopalakrishnan, Xu, Zhong, & Rotello, 2021).

The polysaccharides that have been most closely studied for the development of films and coatings are cellulose (and its derivatives), chitosan, starch, pectin, alginate, carrageenan, pullulan and kefiran (Cazón, Velazquez, Ramírez, & Vázquez, 2017) (Amin et al., 2021) (Chen et al., 2021). Packaging made from these polymers is colourless, tough, transparent, and elastic, with good mechanical and barrier properties (Ribeiro et al., 2021) (Amin et al., 2021) (Cazón et al., 2017) (Vieira, Da Silva, Dos Santos, & Beppu, 2011). However, these materials are quite hydrophilic, which makes them poor barriers to water vapour (Cazón et al., 2017) (Hellebois et al., 2020).

Lipids come from different sources, such as animal and vegetable native oils and fats (peanut, coconut, cocoa, milk butters, jojoba seeds, etc.) (Barbosa, Andrade, Vilarinho, Fernando, & Silva, 2021) (Debeaufort & Voilley, 2009). They are generally small hydrophobic molecules. The main characteristic of packaging developed using lipids as a matrix is that they are very effective at reducing water vapour permeability due to their hydrophobic nature (Debeaufort & Voilley, 2009) but they show low mechanical strength, organoleptic quality reduction (rancidity) and low transparency (Barbosa et al., 2021) (Amin et al., 2021). For these reasons, lipids are usually added as an extra layer or in the form of an emulsion to packaging made with other matrices, such as proteins and polysaccharides (Aguirre-Joya et al. 2016). These combinations are known as composites and they take advantage of the different functional characteristics of each compound while minimizing the disadvantages of each one of them (Ribeiro et al., 2021). In general, a combination of layers of hydrophilic biopolymers (proteins and polysaccharides) with hydrophobic biopolymers (lipids) is produced (Vargas, Pastor, Chiralt, McClements, & González-Martínez, 2008). The addition of a lipid layer improves the water vapour resistance and protein/polysaccharide layers provide integrity, structural cohesion and selective permeability to O_2 and CO_2 (Vargas et al., 2008).

In addition to the biopolymers necessary to form the matrix of the edible films and coatings, other food grade additives can also be added, such as plasticizers, antioxidants, antimicrobials, antifungals, surfactants, natural pigments, etc. (Parreidt et al., 2018) (Hellebois et al., 2020).

3. Probiotics and prebiotics in edible films and coatings

3.1. Probiotics

In 2002, the WHO and FAO defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Food and Agriculture Organization (FAO) of the United Nations, 2002). In recent years certain nuances have been added, depending on their functionality, so they can be re-defined as true probiotics (when microorganisms are viable and active), pseudoprobiotics (if they are viable but inactive) and ghost probiotics (when they are not viable) (Zendeboodi et al., 2020). Among the characteristics of their relationship with the human host that microorganisms must possess in order to be considered probiotics are: (i) being resistant to gastric acids and bile acids, (ii) being able to adhere to mucus and/or human epithelial cells, (iii) having antimicrobial activity against

Table 1
Common probiotics used in edible films and coatings and the 5 last year’s research the latest research in which they have been employed.

Probiotic genus	Probiotic species	Type of edible packaging to which they have been added	Concentration used	Reference
Lactobacillus	*L. casei*	Edible biofilms based on whey protein isolate	0.5 % (*w/v*)	(Dianin, Oliveira, Pimentel, Hernandes, & Costa, 2019)
		Edible coating based on chitosan, alginate and carboxymethyl cellulose	$> 10^7$ CFU/mL	(El-Sayed, El-Sayed, Mabrouk, Nawwar, & Youssef, 2021)
		Edible coating based on whey protein isolates	10^9 CFU/mL	(Odila Pereira, Soares, J.P. Monteiro, Gomes, & Pintado, 2018)
		Edible films based on carboxymethyl cellulose-sodium caseinate	10^9 CFU/mL	(Mozaffarzogh, Misaghi, Shahbazi, & Kamkar, 2020)
		Edible films based on duck feet gelatine	$\sim 10^{10}$ CFU/g film-forming solution-dry basis	(Abedinia et al., 2021)
		Edible films based on citrus pectin	10^9-10^{10} CFU/mL	(Nisar et al., 2022)
	L. rhamnosus	Alginate-based coatings	10^9 CFU/mL	(Bambace, Alvarez, & Moreira 2019)
		Edible films based on carboxymethyl cellulose-sodium caseinate	10^9 CFU/mL	(Mozaffarzogh et al., 2020)
		Edible films based on citrus pectin	10^9-10^{10} CFU/mL	(Nisar et al., 2022)
	L. salivarius	Edible coating based on gelatine additivated with inulin	10^{10} CFU/mL	(Monteiro et al., 2022)
		Spray-coating based on milk powder and sucrose	10^8 CFU/mL	(Wang, Lin, & Zhong, 2021)
	L. plantarum	Bilayer edible coating containing carboxymethyl cellulose in the primary coating and zein in the secondary	8.78 ± 0.10 log CFU/ mL	(Wong, Mak, & Li 2021)
		Edible film based on delipidated egg yolk protein	10^8 CFU/mL	(Sáez-Orviz, Marcet, Rendueles & Díaz 2021)
		Edible coatings based on sodium alginate and prebiotics	10^9 CFU/mL	(Sáez-Orviz, Puertas, Marcet, Rendueles, & Díaz, 2020)
		Edible films based on alginate	10^9 CFU/mL	(Pavli et al., 2017)
		Nanocomposite film based on whey protein isolate and polydextrose	10^9 CFU/mL	(Karimi, Alizadeh, Almasi, & Hanifian, 2020)
		Nanocomposite film based on carboxymethyl cellulose and inulin	$\sim 10^9$ CFU/mL	(Zabihollahi, Alizadeh, Almasi, Hanifian, & Hamishekar, 2020)
	L. acidophilus	Edible coating based on chitosan, alginate and carboxymethyl cellulose	$> 10^7$ CFU/mL	(El-Sayed et al., 2021)
		Edible films based on carboxymethyl cellulose-sodium caseinate	10^9 CFU/mL	(Mozaffarzogh et al., 2020)
		Edible films based on citrus pectin	10^9-10^{10} CFU/mL	(Nisar et al., 2022)
		Edible coating based on sodium alginate	7.36 log CFU/g	(Shigematsu et al., 2018)
		Edible coatings based on sodium alginate, whey, and glycerol	90 mg of dry bacteria/ 10 mL of film-forming solution	(Gregirchak, Stabnikova, & Stabnikov 2020)
	L. pentosus	Edible films based on alginate	10^9 CFU/mL	(Pavli et al., 2017)
		Emulsion film based on gelatine/polydextrose/camellia oil	10^9 CFU/mL	(Zong et al., 2021)
	L. reuteri	Edible films based on carboxymethyl cellulose-sodium caseinate	10^9 CFU/mL	(Mozaffarzogh et al., 2020)
	L. paracasei	Hydrogels of soy protein isolate and sugar beet pectin	10^{10} CFU/mL	(Yan et al., 2021)
		Edible films based on chitosan and *Aloe vera*	2.8×10^9 CFU/mL	(Barragán-Menéndez et al., 2020)
		Edible coating based on pectin	1.5×10^9 CFU/g of coated apple	(Valerio et al., 2020)
Bifidobacterium	*B. lactis*	Edible coating based on chitosan, alginate and carboxymethyl cellulose	$> 10^7$ CFU/mL	(El-Sayed et al., 2021)
		Edible coatings based on alginate, glycerol, inulin and oligofructose	5×10^{11} CFU/mL	(Alvarez, Bambace, Quintana, Gomez-Zavaglia, & Moreira, 2021)
	B. animalis	Edible coating based on whey protein isolates	10^9 CFU/mL	(Pereira et al. 2018)
		Edible films based on alginate or whey protein and prebiotics	10^9 CFU/mL	(Pereira et al., 2019)
	B. bifidum	Edible films based on carboxymethyl cellulose-sodium caseinate	10^9 CFU/mL	(Mozaffarzogh et al., 2020)
		Edible films based on citrus pectin	10^9-10^{10} CFU/mL	(Nisar et al., 2022)
Bacillus	*B. coagulans*	Milk protein concentrate based edible films	10^7 CFU/mL	(Gholam-Zhiyan, Amiri, Rezazadeh-Bari, & Pirsa, 2021)
	B. coagulans	Alginate edible films with bacterial cellulose nanocrystal-stabilized palm oil Pickering emulsion	10^8 CFU/g	(Medeiros et al., 2022)
Saccharomyces	*S. boulardii*	Gelatin and low methoxyl pectin edible films	10^9 CFU/g	(Khodaei, Hamidi-Esfahani, & Lacroix, 2020)
	S. boulardii	Coatings based on acacia gum, modified starch, maltodextrin	10^7 CFU/g	(Singu, Bhushette, & Annapure, 2020)
Kluyveromyces	*K. marxianus*	Edible film based on whey proteins and polysaccharide kefiran	10^6 CFU/cm^2	(Gagliarini, Diosma, Garrote, Abraham, & Piermaria, 2019)

potentially pathogenic bacteria, (iv) having the ability to reduce the adhesion of these pathogens to surfaces and (v) having bile salt hydrolase activity (Food and Agriculture Organization (FAO) of the United Nations, 2002). Some of the common beneficial effects on the host are an improvement in the response of the immune system of the GIT, prevention against infections and strengthening of the intestinal barrier, colonization resistance and the regulation of intestinal transit, among others (Zendeboodi et al., 2020) (Espitia et al., 2016) (Hellebois et al., 2020). The probiotics commonly used in the food industry belong to the group of lactic acid bacteria (LAB) (Zendeboodi et al., 2020) (Guimarães, Abrunhosa, Pastrana, & Cerqueira, 2018). LAB have been allotted the category of "qualified presumption of safety" (QPS) by the EFSA (European Food Safety Authority, European Union (EU) (Barlow et al., 2007)) or "generally recognized as safe" (GRAS) by the FDA (Food and Drug Administration, USA (FDA. *Code of Federal Regulations, title 21, CFR 20, 25, 170, 184, 186 and 570. Substances Generally Recognized as Safe.*, 2016)), allowing their use in food products. The most common probiotics used in edible films and coatings and the latest research in which they have been employed are shown in Table 1.

When probiotics are added to food matrices or edible films or coatings, they must be able to survive passage through the digestive system until they reach the lower GIT, where they will proliferate. Probiotics are only effective if the dosage is sufficiently high. Although there is no scientific consensus on the concentration required to obtain beneficial health effects, some researchers have suggested a minimum amount of between 10^8 and 10^9 CFU per day, but these bacteria have to reach the lower GIT (Espitia et al., 2016) (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). One of the advantages of adding probiotics to edible films and coatings is that this can help them survive the effects of stomach acids and bile salts, increasing their survival rate. This phenomenon has been observed in numerous investigations in which *in vitro* digestion tests have been carried out (Alvarez, Bambace, Quintana, Gomez-Zavaglia, & Moreira, 2021) (Sáez-Orviz, Marcet, Rendueles & Díaz 2021) (Sáez-Orviz, Puertas, Marcet, Rendueles, & Díaz, 2020) (Soukoulis et al., 2014) (Valerio et al., 2020). Probiotics has been added to the formulation of edible films and coatings, to obtain functional packaging and have been employed in numerous food products. Standing out among these products are fruits (Bambace, Alvarez, & Moreira, 2019) (Monteiro et al., 2022) (Wong, Mak, & Li, 2021), vegetables (Shigematsu et al., 2018) (Dianin, Oliveira, Pimentel, Hernandes, & Costa, 2019) and dairy (El-Sayed, El-Sayed, Mabrouk, Nawwar, & Youssef, 2021) (Sáez-Orviz et al., 2020) (Angiolillo, Conte, Faccia, Zambrini, & Nobile, 2014), bakery (Soukoulis et al., 2014) (Gregirchak, Stabnikova, & Stabnikov, 2020), meat (Pavli et al., 2017) (Pereira et al. 2018) and fish products (Mozaffarzogh, Misaghi, Shahbazi, & Kamkar, 2020) (López de Lacey, López-Caballero, & Montero, 2014). The interactions between the matrices, the packaging and the probiotics are decisive in determining their viability and another factor that influences the viability of probiotics is the presence of other compounds, such as prebiotics.

3.2. Prebiotics

The concept of a prebiotic was first introduced by Gibson and Roberfroid in 1995. Currently, it is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). Some of the benefits conferred by prebiotics are the stimulation of the immune system of the GIT, the inhibition of the growth of pathogens in the GIT and the reduction of blood lipids, among others (Gibson et al., 2017).

For a compound to be considered a prebiotic, it must meet certain requirements. These include (i) the resistance to gastric acids and host enzymes, (ii) to be selectively fermented by large intestinal microbiota, and (iii) to have a selective effect on the microbiota, resulting in health-promoting effects on the host (Gibson et al., 2017) (Paulo et al., 2021). The dietary prebiotics most extensively documented to have health benefits in humans and dominant in the market are galactans (such as galacto-oligosaccharides (GOS)), fructans (such as fructo-oligosaccharides (FOS) and inulin) and lactulose (Gibson et al., 2017) (Paulo et al., 2021). Other compounds have also been considered to be prebiotics, such as *xylo*-oligosaccharides (XOS), isomalto-oligosaccharides (IMO), soya-oligosaccharides (SOS), pyrodextrins, dietary fibres, resistant starches, conjugated linoleic acid (CLA), polyunsaturated fatty acids (PUFAs), phenolics, phytochemicals and other non-digestible oligosaccharides (FAO, 2008) (Gibson et al., 2017), although there is still no scientific consensus about whether to add them to the prebiotic category (Bindels, Delzenne, Cani, & Walter, 2015).

A combination of prebiotics and probiotics is described as "synbiotic". Both compounds together have a synergistic behaviour (Figueroa-González, Quijano, Ramírez, & Cruz-Guerrero, 2011). The presence of prebiotics in edible films and coatings allows the viability of probiotics to be improved and several authors have observed their effectiveness in maintaining viability during storage. Bambace et al. (2019) observed an improvement in the viability of *L. rhamnosus* CECT 8361 when inulin and FOS (80 g of each prebiotic/ kg of solution) were added to the sodium alginate coating, with counts above 6.2 log CFU/g during 21 days of analysis. Zabihollahi et al. (2020) developed a carboxymethyl cellulose (CMC) film additivated with *L. plantarum* ATCC® 14917^{TM} and inulin (10 and 20 g/100 g CMC). The presence of the prebiotic meaningfully increased the viable cell numbers of the probiotic with no difference between the concentrations of inulin employed. Similar results were obtained by Pereira et al. (2019). These authors developed edible films based on alginate and whey protein with *B. lactis* BB-12 and different prebiotics (inulin and FOS). The viability improved with the presence of both prebiotics, but the best result was obtained with inulin (2 %, *w/v*), which maintained the viability of the probiotic above 7 log CFU/g during 60 days of storage at 23 °C. Besides, the presence of prebiotics in edible films and coatings also improves viability during *in vitro* digestion tests. Recently, Orozco-Parra, Mejía, & Villa (2020) developed an edible film based on cassava starch additivated with inulin (0.5 %, *w/v*) and *L. casei*. They observed that inulin was capable of reducing the loss of viability of the probiotic in an *in vitro* digestion test. Similar results were obtained by Sáez-Orviz et al. (2020). These authors developed sodium alginate coatings additivated with *L. plantarum* CECT 9567 and lactobionic acid (20 and 40 g/L) as prebiotic. Results showed that the presence of the prebiotic increased the survival of the probiotic by about 11 % after the simulated *in vitro* digestion test. Therefore, synbiotics are a very promising area for the development of new packaging and functional foods.

4. Formation mechanisms of edible films and coatings

There are three different mechanisms of edible film formation. In the case of simple coacervation, the phase change or the precipitation of the hydrocolloid can occur through three different phenomena: (i) the solvent evaporation process, (ii) the incorporation of a non-electrolyte compound (in which the hydrocolloid is not soluble) or (iii) the incorporation of an electrolyte compound (Guilbert, Gontard, & Gorris, 1996) (Parreidt et al., 2018). In the latter case, the electrolyte can be obtained by adjustment of the pH of the film-forming solution, which promotes cross-linking (Ribeiro et al., 2021). These three phenomena lead to an increase in the concentration of the biopolymer, resulting in molecular aggregation and the formation of a three-dimensional network (Khwaldia, Ferez, Banon, Desobry, & Hardy, 2004). In complex coacervation, the interaction or precipitation occurs when two hydrocolloid solutions with opposite electron charges are mixed (Guilbert et al., 1996) (Parreidt et al., 2018). Although there are different parameters that can be modified in the process, such as pH, temperature or concentration of the biopolymers (Warnakulasuriya & Nickerson, 2018), the most important is the charge density (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). The last mechanism is gelation or thermal coagulation. In this case, interaction or precipitation of the hydrocolloid is achieved by heating the forming-solution, so causing the denaturation, gelification and or precipitation of the compound (as occurs when denaturing certain proteins such as ovalbumin (Guilbert et al., 1996) or soy protein (Khwaldia et al., 2004)) or by rapid cooling of the forming-solution, resulting in a gelling phenomenon (as occurs with agar or gelatine) (Parreidt et al., 2018) (Umaraw & Verma, 2017).

5. Edible film and coating production methods

Edible films and edible coatings represent different packaging concepts. Edible films are materials that, once dried, can be applied directly to foods as wrapping or covering materials. However, edible coatings are applied to food products as fluid liquids or gels (Hellebois et al., 2020) and form a thin layer on the surface of foodstuff (Maringgal, Hashim, Mohamed Amin Tawakkal, & Muda Mohamed, 2020). In both cases, one of the aims of the edible packaging is to extend the shelf-life of food by creating a barrier between the foodstuff and the environment and the film or coating is then consumed as part of the whole product (Maringgal et al., 2020) (Pop et al., 2020).

5.1. Edible film production methods

The two different methods of producing edible films are shown in Fig. 1. The solvent casting method requires the solubilisation or dispersion of the biopolymers in a solvent followed by the drying of the film-forming solution. It has three steps (Fig. 1-A). The first step is the solubilisation phase, in which the biomaterials, plasticisers, bioactive compounds and other additives that may be added are dissolved in a suitable solvent. To avoid the formation of bubbles, the film-forming solution may be subjected to sonication, centrifugation and/or vacuum processing before the second step. The second stage consists of casting, in which a fixed amount of film-forming solution is poured onto a flat, level surface. The last step is the drying phase, in which the evaporation of the solvent occurs. After this last stage and once all the solvent has evaporated, the film should be peeled easily from the surface without breaking (Rhim, Mohanty, Singh, & Ng, 2006) (Parreidt et al., 2018) (Ribeiro et al., 2021) (Chen et al., 2021). Due to its simplicity, as no specialized equipment is needed and the cost is low, this is the most frequently employed film-forming technique at laboratory and pilot scales (Suhag, Kumar, Petkoska, & Upadhyay, 2020) (Parreidt et al., 2018). However, this method has two disadvantages for scaling up to an industrial level: it is not suitable for creating films much larger than 25–30 cm and a large amount of water needs to be evaporated, so the drying times are long, which makes the process expensive (De Moraes, Scheibe, Sereno, & Laurindo, 2013) (Jeevahan et al. 2020). In an attempt to alleviate some of these problems, the tape casting technique (Fig. 1-B), which is common for other production processes such as paper, plastics, ceramics, and industrial paints (Boch & Chartier 1998), has been adapted for edible film production. With the tape casting technique, films can be obtained continuously with shorter drying times than with the casting solvent technique (De Moraes et al., 2013).

The dry methods are based on the thermoplastic properties of the polymers. In this case, the film-forming solution, which has a low amount of water or solvent and includes the plasticizer compound, such as glycol or sorbitol, is heated above its glass transition temperature (Parreidt et al., 2018) (Ribeiro et al., 2021) (Chen et al., 2021) (Suhag et al., 2020). This leads to the conversion of the film-forming solution into an elastic state, due to the thermoplastic behaviour of proteins at low moisture levels (Gómez-Guillén et al., 2009). After a compression and/or cooling process, a thin layer of film is formed. The most common methods in dry processes are extrusion (Fig. 1-C) and compression methods. Extrusion processes have been widely used to produce conventional plastics (García, Gómez-Guillén, López-Caballero and Barbosa-Cánovas 2016). They can be divided into three stages. The first is the preparation of the film-forming solution and its introduction in the feeding zone. This is followed by kneading, where the mixture is compressed and the pressure, temperature and density of the film-forming solution are increased. The last stage is the heating stage, where the film-forming solution attains the appropriate final characteristics. The film-forming solution is extruded through a nozzle at a defined speed and, finally, the films are dried (García, Gómez-Guillén, López-Caballero and Barbosa-Cánovas 2016) (Suhag et al., 2020) (Chen et al., 2021). Extrusion processes are very promising processes which have generated great interest and offer the possibility of easy scale up to produce edible films by a continuous process.

Compared to wet methods, the dry methods have several advantages, such as low energy consumption (fewer evaporation steps are needed) and short processing times (Suhag et al., 2020) (Parreidt et al., 2018). Moreover, these techniques use very little solvent (Liu, Xie, Yu, Chen, & Li, 2009). In addition, several authors have observed an improvement in certain mechanical and optical properties when compared to the casting solvent method (Rhim et al., 2006) (Andreuccetti et al., 2012) (Ochoa-Yepes, Di Giogio, Goyanes, Mauri, & Famá, 2019). Finally, dry techniques make it possible to obtain more diverse shapes than wet methods (Suhag et al., 2020) and extrusion methods can be implemented as a continuous unit process (Gómez-Guillén et al., 2009).

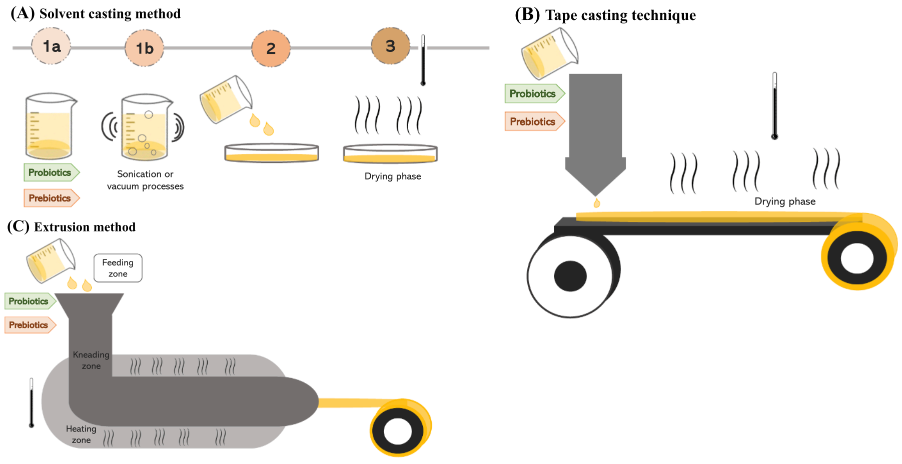

Fig. 1. *(A)* Schematic diagram of the solvent casting method. 1a- Preparation of the film-forming solution. 1b- Sonication or vacuum processes may be necessary to remove bubbles. 2- Casting on a plate. 3- Drying phase in a hot air chamber or an oven. *(B)* Schematic diagram of the tape casting technique, as a continuous process similar as the solvent casting method. *(C)* Schematic diagram of the extrusion method. In all cases, prebiotics and probiotics are added to the film-forming solution.

5.2. Coating production methods

Regarding the use of coatings, the two most important factors to consider are the method of application and the ability of the coating to adhere to the surface of the food product (Parreidt et al., 2018). The two usual methods are spraying and dipping (Fig. 2).

Dipping is the most common method (Suhag et al., 2020) and consists of three steps (Fig. 2-A). First, the piece of food is immersed in the coat-forming solution during a certain period of time. In some cases, it is necessary to repeat this step with another solution that allows better cross-linking of the coating (Ribeiro et al., 2021), e.g., first a sodium alginate coating solution followed by an immersion in a $CaCl_2$ solution (Parreidt et al., 2018). Then, the excess solution is removed by deposition (Suhag et al., 2020). Finally, the product is submitted to a drying process (Parreidt et al., 2018) (Ju et al., 2019), which could be at room temperature or include heating (Suhag et al., 2020). After the drying period, a layer of coating is formed on the surface of the product. However, the thickness of the layer formed may not be homogeneous and thick coatings may form (Ju et al., 2019). This method has several advantages, such as the process being generally simple, short, and low cost. For these reasons, it is the most common method used at laboratory scale (Atieno, Owino, Ateka, & Ambuko, 2019). Besides, it allows the piece of food to be completely covered, regardless of its shape or structure (Parreidt et al., 2018). That is why the dipping method has been used in different types of food products such as fruits (Strano et al., 2021) (Saleem et al., 2020) (Saleem et al., 2021) (Wong et al., 2021), vegetables (Divya, Smitha, & Jisha, 2018) (Aisyah, Murlida, & Maulizar, 2022), meat (Abdel-Naeem, Zayed, & Mansour, 2021) (Lashkari, Halabinejad, Rafati, & Namdar, 2020) (Gedikoğlu, 2022), bakery (Eom, Chang, Lee, Choi, & Han, 2018) (Nayanakanthi, Senanayake, & Siranjiv, 2021) and dairy products (Sáez-Orviz et al., 2020) (Jotarkar, Panjagari, Singh, & Arora, 2018) (Siriwardana & Wijesekara, 2021).

The spraying method is the most commonly used in the food industry (Suhag et al., 2020). This method is based on the distribution of the coating solution on the surface of the food in the form of drops with the help of nozzles (Parreidt et al., 2018), a process which is effective thanks to the development of high pressure spray applicators and air atomizing systems (Silva-Vera et al., 2018) (Fig. 2-B). A key requirement of this method is that the viscosity of the coating solution should not be high (Ju et al., 2019). The spraying method has the advantages of forming a coating with uniform thickness over the whole surface of the piece of food and the possibility of applying layers of coatings with different characteristics (Suhag et al., 2020). Besides, the amount of coating solution needed is less, the possibility of contamination is lower and it is possible to work with large food surfaces (Parreidt et al., 2018) (Ribeiro et al., 2021). Some of the recent research using the spraying method is based on studies on fruits (Farina, Passafiume, Tinebra, Palazzolo, & Sortino, 2020) (Jiang et al., 2019) (Lara et al., 2020), meat (Gedarawatte et al., 2021) (Apriliyani et al., 2020), and fish products (Simen Sørbø, 2022) (Kulawik, Jamróz, Zając, Guzik, & Tkaczewska, 2019).

5.3. Incorporation of probiotics and prebiotics in edible films and coatings

In the case of probiotics, regardless of the production method of edible films or coatings, the key factor is maintaining their viability throughout the entire process (Zoghi et al., 2020). During the production processes of edible packaging, probiotics can be subjected to different environmental stresses (Fig. 3), such as high or low temperatures, acid and alkaline pH, oxidative stress, high hydrostatic pressure, osmotic pressure and starvation (Mbye et al., 2020).

In all production methods, the microorganisms are usually added to the film-forming or coat-forming solution, which must have a suitable pH and temperature, depending on the probiotic strain being tested. The most important phases in terms of the viability of the probiotic are the drying stages, where the temperature increases considerably, in addition to the fact that the water activity (a_w) can decrease, compromising the survival of the microorganisms. To mitigate the loss of viability during the drying steps, cytoprotective plasticizers can be used (Hellebois et al.,

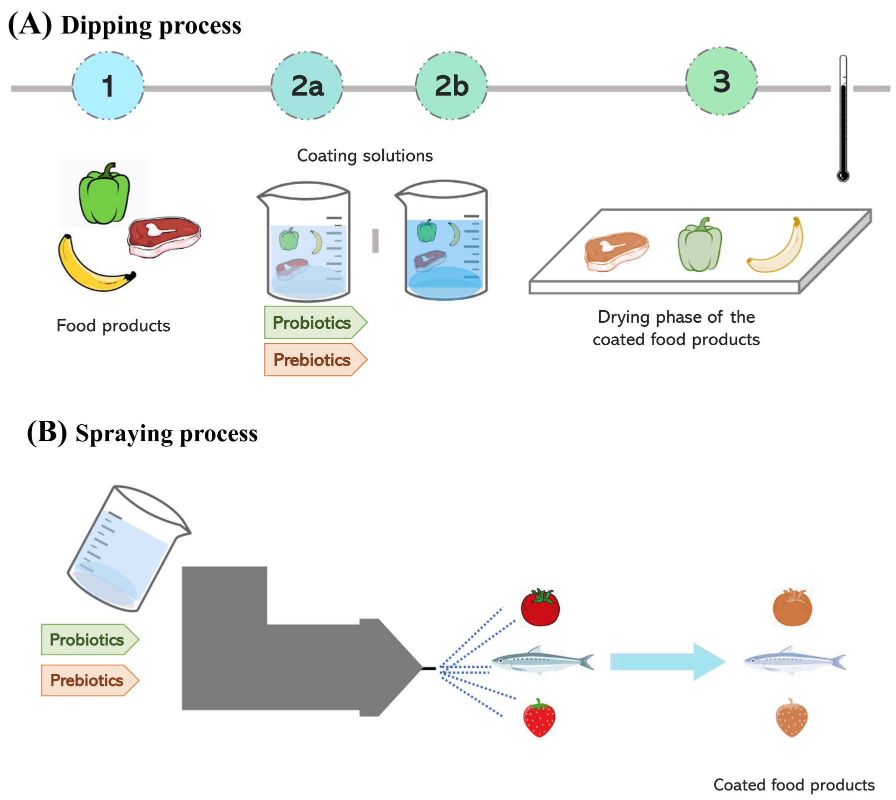

Fig. 2. *(A)* Schematic diagram of the dipping process. 1-Various food products. 2a- Food products are immersed in the coat-forming solution. 2b- In some cases, it is necessary to include the step of immersion in a cross-linking solution. 3-Excess of solution is removed by deposition and drying process can occur at room temperature or by heating. *(B)* Schematic diagram of the spraying process. The coating solution is distributed on the surface of the food pieces from the drops that form in the nozzle of the equipment. In both cases, prebiotics and probiotics are added to the coat-forming solutions.

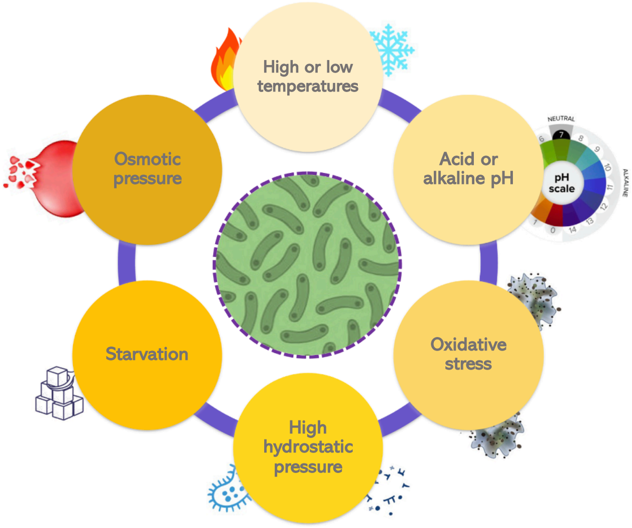

Fig. 3. Key factors to consider when including probiotics in the formulation of edible films and coatings.

2020). Resistance to each of these parameters is strain dependent, so it is important to select appropriately the probiotic to use based on the production method to be employed (Mbye et al., 2020). When developing edible films and coatings with probiotic microorganisms, the most common methods are usually solvent casting or dipping techniques (Hellebois et al., 2020). The encapsulation of microorganisms using techniques such as spray drying and atomization have been extensively studied (Guimarães et al., 2018) (Pop et al., 2020) (Rodrigues, Cedran, Bicas, & Sato, 2020). However, there is very little research on the use of dry methods to make films and coatings with free probiotics. Recently, Wang et al. (2021) developed a novel spray-coating strategy using milk powder and sucrose as matrix and *L. salivarius* as the probiotic microorganism. They observed that a drying temperature of 60 °C for up to 30 min was optimal, while temperatures higher than 63 °C led to deactivation of the bacterium.

In relation to the addition of prebiotics, they are also added to the film-forming or coat-forming solutions. In this case, the parameters that must be taken into account are the temperature, pH, time of solubilization or the method of dispersion (Paulo et al., 2021). The solubilization of the prebiotic will depend on its nature and its compatibility with the chemical characteristics of the film-forming or coat-forming solutions. However, the addition of prebiotics is simpler and implies fewer difficulties than in the case of probiotics, since these are compounds that are not as sensitive to physical and chemical factors, and they are not easily degradable.

6. Interactions between biopolymers in film network and the effect of the presence of probiotics and prebiotics

The different biopolymers that compose the matrix of films and coatings have a variety of functional groups, which leads to a great variety of interactions inside the matrices (Chen et al., 2021). In some cases, these interactions may be more important than the physicochemical properties of the individual biopolymers (Zhang et al., 2021). The interactions that occur inside the matrix can be divided into two main groups: covalent bonding and non-covalent interactions.

Covalent bonding can be defined as strong chemical bonds that involve the sharing of electron pairs between atoms. In this group are different covalent interactions such as esterification, etherification, amidation, glycosylation, etc. Generally, these covalent bonds are formed between amino ($-NH_2$, -NRH), hydroxyl (–OH) and carboxyl ($-CO_2H$) groups in proteins and polysaccharides (Chen et al., 2021) (Zhang et al., 2021). The presence of these types of interactions, their number and their nature are linked to the physical properties of the edible packaging developed. Specifically in the case of a protein matrix, disulphide bonds have a significant impact on the structure and morphology of edible films (Deng et al., 2020). Disulphide bonds can be defined as strong covalent bonds that can be obtained by the oxidation of a pair of thiol groups (Fass & Thorpe, 2018). Recent studies have focused on the use of this type of interaction to modify the characteristics of edible packaging. Kumari et al. (2021) developed a fenugreek protein-based edible film in an alkaline environment (pH 12). At this pH, these authors observed that films were more stable due to the presence of disulphide bonds, and this also produced a reduced WVP in the films they created. Deng et al. (2020) developed a disulphide bond crosslinked gelatin/ε-polylysine edible film. Results showed that the oxidation of thiol groups led to a more compact and denser microstructure, and this resulted in an improvement in the water resistance of this protein film. These results are promising, as they could improve the poor water resistance of films developed exclusively with proteins, since this is their main disadvantage. Covalent crosslinking can also be induced through chemical (alkali treatment, free radical initiated (Chen et al., 2021) and irradiation (Gopalakrishnan et al., 2021)) or enzymatic methods (transglutaminase, laccase and peroxidase, among others (Chen et al., 2021)). Of the chemical methods, irradiation or photocrosslinking is one of the most common (Li et al., 2020a). Ben-Fadhel et al. (2021) developed a calcium caseinate edible coating that was treated with γ- irradiation and observed that γ- irradiation at 32 kGy improved the mechanical and water vapour barrier properties of the calcium caseinate coating. Huang et al. (2020) observed that electron beam irradiation has a positive effect on the tensile strength, opacity values and microstructure of fish gelatine films additivated with antioxidants, due to the formation of new bonds inside the matrix. In the case of enzymatic methods, transglutaminase is one of the enzymes commonly used to improve the characteristics of films using protein as the matrix. Transglutaminase introduces isopeptide bonds between the amino acids glutamine and lysine (Giosafatto, Fusco, Al-Asmar, & Mariniello, 2020). Numerous authors have seen an improvement in the mechanical properties of their films after using this enzyme (Minh et al., 2019) (Ahammed et al., 2021) (Escamilla-García et al., 2019) (Marcet, Sáez, Rendueles, & Díaz, 2017).

As for the non-covalent interactions, they include van der Waals forces, hydrogen bonding, and hydrophobic and electrostatic interactions (Chen et al., 2021) (Zhang et al., 2021). Van der Waals forces do not result from a chemical electronic bond; they are distance-dependent and therefore are weaker. Hydrogen bonding is an electrostatic interaction between hydrogen atoms and negatively charged atoms in which there is always a hydrogen donor and a hydrogen acceptor (Zhang et al., 2021). Hydrogen bonding interactions are very common within film and coating matrices and several authors have observed their importance by using Fourier transform infrared (FTIR). Dai, Zhang and Cheng (2019) verified the formation of hydrogen bonds between starch and glycerol in starch-based edible films. This bonding made the polymer matrix more structurally integrated and improved the transparency of the films. Nguyen et al. (2020) observed the formation of hydrogen bonds between polyphenol compounds and chitosan on their edible films. These interactions resulted in an improvement in the water vapour transmission rate of the films, but the high number of interactions that were formed caused a decrease in the film plasticity. Davoodi et al. (2020) developed *Salvia macrosiphon*/chitosan edible films and observed hydrogen-bonded networks by FTIR analysis. Hydrogen-bonding enhanced the mechanical and water vapour barrier properties of their edible films. Thus, there are electrostatic interactions inside the biopolymer matrix due to attractive and repulsive forces between groups, which are caused by their electric charges. In these cases, the charge depends on the pH of the system and the presence of salts can affect the net charge (Zhang et al., 2021).

Understanding all these interactions, how they occur and how they can be modified is of vital importance to improve the physical and mechanical properties of the edible films and coatings developed. Besides, all the interactions mentioned above are affected when probiotics and prebiotics are added to the biopolymer matrix.

6.1. Influence of probiotics in the interactions

All the aforementioned non-covalent and covalent interactions are affected by the presence of probiotic microorganisms in the matrix of the packaging. Although some studies have been carried out, the mechanisms and biochemical phenomena related to how probiotics are stabilized in the biopolymer matrices are not yet known (Abedinia et al., 2021) (Soukoulis, Behboudi-Jobbehdar, Macnaughtan, Parmenter, & Fisk, 2017). One of the possible ways of interaction or stabilization between the probiotic cells and the matrix would be through hydrogen bonds via the polar heads of the phospholipid membranes of microorganisms (Abedinia et al., 2021) (Ma, Jiang, Ahmed, Qin, & Liu, 2019). These interactions between the microorganisms and the biopolymers cause changes in the rest of the interactions of the matrix, changing the physical and chemical properties of the edible packaging. One of the changes that arises when adding microorganisms to the biopolymer matrix is steric hindrance. The probiotic cells occupy a space in the matrix that can prevent some interactions, both covalent and non-covalent, from taking place. Several authors have noted changes in matrix properties due to this factor. In some cases, certain properties have been improved by the presence of microorganisms, while in others they have been worsened. Table 2 shows examples of some changes that have been analysed recently by several authors.

Some authors have studied this topic recently. Wai et al. (2022) developed a sodium caseinate edible film with *Limosilactobacillus fermentum* as probiotic bacteria. They observed that the incorporation of probiotics resulted in a reduction of the tensile strength of the films. In this case, the presence of the probiotic could reduce the cohesiveness of the polymer chains, making the interaction weaker. Sogut, Filiz, & Seydim (2022) prepared whey protein isolate, and carrageenan-based edible films as carriers of different probiotic bacteria (*L. acidophilus*, *L. plantarum* and a mixture culture of *Lactobacillus* spp., *Lactococcus* spp. and *Bifidobacterium* spp.). These authors noticed that the incorporation of probiotic bacteria significantly influence the thickness of the edible films. This can be explained by the steric hindrance and by a higher water-holding capacity during the drying process. They also observed differences in the microstructure of the films, as they had a higher number of holes due to the embedment of bacterial cells. As a result, these films had higher WVP values and lower tensile strength and elasticity. Semwal, Ambatipudi, & Navani (2022) made sodium caseinate films with chia mucilage as a protectant for the incorporation of *L. fermentum* NKN51 and *L. brevis* NKN52. They found that the addition of probiotic bacteria significantly reduced the solubility of the films. This low solubility could be explained as a reduction of the pH to 5–5.5 due to the metabolic end products (lactic acid) of the probiotic bacteria inside the films. In this case, as they used sodium caseinate as matrix of the films, when the pH came closer to the isoelectric point of casein (4.6), the electrostatic repulsion is lower, resulting in modified protein's molecular conformation and in an stronger protein–protein interaction, resulting in a lower solubility. Yan et al. (2021) developed soy protein hydrogels with sugar beet pectin additivated with *L. paracasei* LS14. The presence of the probiotic weakened the hardness of the hydrogels, due to the steric hindrance effect, as this prevented the formation of ordered intermolecular aggregates during the gelation process. In addition, they observed that probiotic cells caused changes in the tertiary structure of the proteins. Ma et al. (2019) developed edible films with three different film-forming solutions (sodium alginate, sodium carboxymethylcellulose and collagen) additivated with *L. lactis*. These authors observed changes in WVP values. These values increased when the microorganisms were added to the films, which could have happened because the addition of bacteria would change the spatial structure of the molecules, reducing intermolecular interactions and increasing the intermolecular space, thus leading to an increase in permeability to water vapour. Karimi et al. (2020) prepared films with whey protein, polydextrose and nanocellulose fibres additivated with *L. plantarum*. The authors attributed the good viability of the probiotic inside the matrix to the presence of nutrients and free radical scavenging agents, and in addition, to the interaction between polydextrose and the phospholipids of the bacterial membrane. Otherwise, Li et al. (2020b) observed differences in a cassava starch/carboxymethylcellulose matrix depending on the microorganism they added. *L. plantarum* achieved a good dispersion within the matrix, although the probiotic cells were found as discontinuous particles in the matrix, inhibiting the migration of the polymer chain. This strain improved WVP film properties. However, *Pediococcus pentosaceus* did not achieve a homogeneous distribution within the matrix, as observed from morphological analysis, which caused the films to have poor WVP and mechanical properties. Shahrampour et al. (2020) concluded that the compatibility between the probiotic microorganism and the biopolymers of matrix employed is a key factor. They developed alginate/pectin edible films containing *L. plantarum* KMC 45. The SEM images showed that the probiotic cells had not modified the microstructure of the films, which indicated a good compatibility between alginate and pectin. Furthermore, the addition of the probiotic cells partially improved the mechanical properties and the WVP. These authors suggested that, in this specific case, the bacteria were able to reduce the intermolecular space due to hydrogen bonding with the film-forming agent.

From these studies, it can be concluded that the interactions that take place in the edible packaging when probiotic cells are added depend both on the type of microorganism that is added and on the type of biopolymer of the matrix, as well as the compatibility between them. Most of the research that has been done focuses on the viability of probiotics within films and coatings and not on the interactions that occur. A better understanding of the mechanisms of interaction and stabilization of microorganisms will provide more information on how to improve the viability of probiotics within edible packaging.

6.2. Influence of prebiotics on the interactions

Adding prebiotic compounds influences the interactions that will occur between the biopolymers inside the packaging matrix. The interactions will be reduced or reinforced, depending on the nature of the prebiotic compounds and their compatibility with the biopolymers of the matrix. The type of changes that occur in the matrices when adding different kind prebiotics have been studied by several authors. Recent research is shown in Table 3.

Several studies have focused on the interactions of oligosaccharides in edible packaging. Ceylan & Atasoy (2022) developed films with sodium caseinate as matrix and inulin and FOS as prebiotic compounds (0, 1, 2 and 3 %, *w/v*). They observed that films were thicker as the concentration of prebiotics was higher. This could be explained as an increase in the dry matter as the volume kept constant. Furthermore, the presence of inulin and FOS increase the moisture content. This increase may be due to the individual water holding capacity of both prebiotics and their hygroscopic capacity help to retain water in the film matrix. In the same way, the addition of inulin and FOS increased polymeric chain mobility, leading to a less resistant film formation and lower tensile strength values. One interesting result they found was in relation to the opacity of the films. Results obtained were different depending on the type of prebiotic. The molecular size of prebiotics affects water solubility and they can increase light transmittance properties. Seyedzadeh-hashemi et al. (2022) prepared carboxymethyl cellulose/β-glucan-based films with inulin (2 and 4 %) as prebiotic compound. The incorporation of inulin led to lower tensile strength values because of the plasticizing effect of prebiotics. Inside the matrix may be unstable interactions between the biopolymer and inulin instead of strong biopolymer-biopolymer interactions. These authors observed by SEM images that the inclusion of inulin brought heterogeneity, bigger cracks and rougher

Table 2
Studies from the last 3 years about changes observed when probiotics were added to the matrix of edible films and coatings.

Probiotic strain	Biopolymers employed as matrix in the edible packaging	Changes observed due to the presence of probiotics	Reference
L. paracasei LS14	Soy protein and sugar beet pectin	• Weaker hydrogels in terms of firmness Enhancement of the swelling ratios Avoidance of the formation of ordered intermolecular aggregates during the gelation process Disruption of the microstructure of the hydrogels	(Yan et al., 2021)
Lactococcus lactis ATCC 11,454	Sodium alginate, sodium carboxymethylcellulose, collagen and glycerol as plasticizer	• Alterations in the colour and lustre of the edible films Higher WVP values	(Ma, Jiang, Ahmed, Qin, & Liu, 2019)
L. plantarum PTCC 1058	Whey protein, polydextrose, and nanocellulose fibres	• No significant changes in physical and mechanical properties The interaction between the phospholipids of the bacterial membrane with polydextrose increased their viability	(Karimi, Alizadeh, Almasi, & Hanifian, 2020)
L. plantarum	Cassava starch, carboxymethylcellulose, and glycerol	• Decrease in the light transmittance of the film Achievement of good dispersion inside the matrix by morphological analysis Improvement of the WVP properties	
Pediococcus pentosaceus		• Decrease in the light transmittance of the film Did not achieve a good dispersion inside the matrix by morphological analysis Higher values of WVP Poor mechanical properties	(Li et al. 2020b)
L. plantarum KMC 45	Alginate, pectin, and glycerol and sorbitol as plasticizers	• No modification of the microstructure that showed good compatibility between alginate and pectin Partial improvement in the mechanical properties and WVP.	(Shahrampour, Khomeiri, Razavi, & Kashiri, 2020)
L. casei 01	Whey protein isolate and glycerol	• Edible films with probiotics were thicker They showed a higher solubility Regarding mechanical properties, they were less flexible	(Dianin, Oliveira, Pimentel, Hernandes, & Costa, 2019)
L. casei DN-114001*, B. bifidum* DSMZ 20215*, L. acidophilus* DSM 20,079 and *L. rhamnosus* GG E-96666	Citrus pectin and glycerol as plasticizer	• Slight decrease in the swelling index Increase in the WVP Reduction on the clearness of the films Reduction in the tensile properties of the films	(Nisar et al., 2022)
B. lactis B-1922*, L. acidophilus* CH-2 and *L. casei* B-1922	Chitosan, sodium alginate and carboxymethyl cellulose, and glycerol as plasticizer	• Changes in the microstructure of sodium alginate films due to the aggregation of probiotics Changes in water vapour transmission rate (WVTR) and water solubility values	(El-Sayed, El-Sayed, Mabrouk, Nawwar, & Youssef, 2021)
L. plantarum CECT 9567	Egg yolk protein and glycerol as plasticizer Lactobionic acid as prebiotic	• Changes in mechanical properties. Puncture deformation values were lower when probiotic was in the film matrix	(Sáez-Orviz, Marcet, Rendueles & Díaz 2021)
L. plantarum	Sodium alginate and glycerol as plasticizer	• Higher thickness values Increase in the WVP values	(Akman, Bozkurt, Dogan, Tornuk, & Tamturk, 2021)
Limosilactobacillus fermentum	Chitosan and sodium caseinate	• Significant decrease in tensile strength values Significant reduction in Young's modulus values	(Wai et al., 2022)
Enterococcus faecium FM11-2	Cactus mucilage (*Opuntia ficus-indica*), gelatine and plasticizer (glycerol or sorbitol)	• Reduction of the weight loss Regarding mechanical properties, a decrease in elongation at break	(Todhanakasem et al., 2022)
L. acidophilus DSM 20079, *L. plantarum* and a mixture of *Lactobacillus* spp., *Lactococcus* spp. and *Bifidobacterium* spp.	Whey protein isolate, carrageenan and glycerol	• Mixed culture-added films had higher thickness values Slightly decrease in the flexibility of films WVP values increased with the incorporation of probiotic bacteria Slightly increase in the opacity of films	(Sogut, Filiz, & Seydim, 2022)
L. fermentum NKN51 and *L. brevis* NKN72	Sodium caseinate, mucilage from Chia seed and glycerol	• Probiotic cells did not significantly influence the thickness of the films The addition of probiotics reduced the solubility of the films Bacterial cells enhanced Young's modulus values of the films	(Semwal, Ambatipudi, & Navani, 2022)
L. plantarum IS-10506	Sodium alginate and glycerol	• Improvement of the performance to light transmission Better water barrier properties	(Wardana, Wigati, Tanaka, Tanaka, & Surono, 2022)

Table 3
Recent studies about changes observed when prebiotics were added to the matrix of edible films and coatings.

Prebiotic compound	Biopolymers employed as matrix in the edible packaging	Changes observed due to the presence of prebiotics	Reference
Inulin (0.1, 0.5 and 1 %, *w/v*)	Cassava starch and glycerol as plasticizer	• Decrease in tensile strength Increase in elongation at break Increase in WVP values and water solubility	(Orozco-Parra, Mejía, & Villa, 2020)
Inulin (2 %, *w/v)*	Alginate and whey protein and glycerol as plasticizer	• Decrease in the tensile strength of the films	(Pereira et al., 2019)
Inulin (10 and 20 g/100 g of dry film)	Carboxymethyl cellulose, cellulose nanofiber and glycerol as plasticizer	• The presence of prebiotic increased the thickness of the films Reduction in the moisture absorption values	(Zabihollahi, Alizadeh, Almasi, Hanifian, & Hamishekar, 2020)
FOS (1 g/ 100 mL film-forming solution)	Nanofibrillated bacterial cellulose and cashew gum	• Decrease in tensile strength Films were more permeable to water vapour Increase in the water solubility Rougher surfaces in SEM micrographs	(Oliveira-Alcântara et al., 2020)
FOS (15 g/L)	Linseed mucilage, sodium alginate and glycerol and polysorbate as plasticizers	• Prebiotic contributed to improving the colour of the edible coatings	(Rodrigues, Cedran, & Garcia 2018)
GOS (10, 20 and 30 g/ 100 g)	Whey protein isolate and glycerol as plasticizer	• Decrease in tensile strength and increase in elongation at break at concentrations greater than 20 g/100 g of film Lower diffusion and lower permeability values	(Fernandes et al., 2020)
XOS (10, 20 and 30 g/ 100 g)			
XOS (5, 10, 15 and 20 % of dry weight)	Chitosan, hemicelluloses, and cellulose nanofiber	• Less compact films Less tensile strength Higher WVP values	(Xu, Xia, Yuan, & Sun, 2019)
Inulin and FOS (1, 2 and 3 % *w/v*)	Sodium caseinate and glycerol	• Increase of thickness at higher concentrations of inulin and FOS Increase in moisture content of the films Lower water solubility values at higher concentrations of inulin and FOS Increase of the mobility of the polymeric chain, leading to less resistant film formation	(Ceylan & Atasoy, 2022)
Inulin (2 and 4 %, *w/v*)	Carboxymethyl cellulose, β-glucan and glycerol	• Increase in the thickness of the films Decrease in tensile strength values but increase in elongation at break values Water solubility and moisture content increase continuously and significantly as the inulin concentration increased	(Seyedzadeh-hashemi et al., 2022)

surface, which implied a less number of interaction in the polymeric matrix. Fernandes et al. (2020) studied the influence of the addition of GOS and XOS to whey protein films. These authors found no differences in the microstructure of the films. However, they did observe differences in the mechanical properties, identifying a decrease in tensile strength and an increase in elongation at break, depending on the concentration of added prebiotic (at concentrations greater than 20 g /100 g of film). This change in properties may be due to reduced formation of strong protein–protein interactions. Furthermore, the addition of GOS and XOS, with their large size and structures containing sugars and hydroxyl groups, could induce the formation of complex interactions, which would offer more flexibility to these structures. Regarding the WVP properties, the films with prebiotics showed lower diffusion and lower permeability, probably because these molecules lengthen and hinder the path that water vapour molecules must follow. Furthermore, water interacts with GOS and XOS molecules, due to their characteristics, forming strong interactions between water and hydroxyl groups. Xu et al. (2019) developed hemicellulose/chitosan-based films reinforced with cellulose nanofiber and XOS. These authors observed differences in WVP values and mechanical properties. The strong interactions between the biopolymer chains (hydrogen bonds and electrostatic interactions) were disrupted by the presence of XOS, making the films less compact, with lower tensile strength and accelerated permeation of water molecules. Research has also been carried out on inulin. Orozco-Parra et al. (2020) developed an edible film based on cassava starch and inulin. As with the oligosaccharides, inulin decreased the tensile strength of the films and increased their elongation. Prebiotics can have a plasticizing effect by reducing strength while increasing elongation. WVP values increased and so did solubility. Inulin is a very hygroscopic and water-soluble compound, so it has a great affinity for water molecules, increasing the diffusion of water vapour. Pereira et al. (2019) developed edible films based on alginate and whey protein with inulin in their matrix. The only significant differences due to the presence of inulin were in the tensile strength of the films.

From these investigations it can be concluded that prebiotic compounds can have an effect similar to plasticizers in the matrix of biopolymers (Paulo et al., 2021), and therefore, these compounds allow, in general, more flexible films to be produced (Urbizo-Reyes, San Martin-González, Garcia-Bravo, & Liceaga, 2020). Besides, the interactions that take place depend on the compatibility of the nature of the biopolymers with the prebiotic compounds and their concentration. In addition, in some cases they can be used as a reinforcement agent (Paulo et al., 2021).

Regarding the combination of both probiotic microorganisms and prebiotic compounds, a combination of the interactions explained above takes place. The main characteristic in all cases is the increase in the viability of the probiotics during processing and storage of the films and coatings. In the case of inulin, Orozco-Parra et al. (2020) observed a protective effect on the viability of *L. casei* during storage and the same was found by Pereira et al. (2019), since in the films with this prebiotic compound the survival of *B. animalis* improved after 60 days of storage. This effect has been observed in other prebiotic compounds such as FOS. Oliveira-Alcântara et al. (2020) observed a protective effect of FOS on probiotic bacteria during film drying, as synbiotic films exhibited higher bacterial viability that probiotic films.

7. Regulatory aspects in the commercialization of functional packaging

Edible biopolymers and edible packaging have been gaining importance in recent times due to their sustainability and versatility (Amin et al., 2021). The regulations regarding food packaging vary from one country to another (Jeevahan et al. 2020), but generally, as they are edible films and coatings, they must adhere to the regulations related to foodstuffs. Thus, all the compounds used must be recognised as food grade, either as food ingredients or additives, and must be approved by the Codex Alimentarius (Debeaufort & Voilley, 2009) and the corresponding food authorities (i.e., the EFSA in the case of the EU and the FDA in the case of the USA). In the particular case of probiotics, they must be recognised as QPS (Barlow et al., 2007) or GRAS (*FDA. Code of Federal Regulations, title 21, CFR 20, 25, 170, 184, 186 and 570. Substances Generally Recognized as Safe.*, 2016) in order to be used in the food industry. Therefore, synbiotic biomaterials must also adhere to this regulation. However, there are no special guidelines to control the commercialization of biodegradable and functional packaging materials (Amin et al., 2021). In the case of the EU, it is stipulated that all food contact materials must not endanger human health, must not cause intolerable food composition changes, must not cause changes in organoleptic properties, and must be manufactured according to good manufacturing practices (EC No. 1935/2004).

Besides, regulatory approval usually depends on both the formulation and also the application process and these need to be considered separately (Schmid & Müller, 2018). Continuing research in this area will help to better understand the behaviour of edible packaging and its use with foodstuffs and, thus, its regulation.

8. Conclusions and future trends

This review highlights recent research on edible films and coatings additivated with probiotic microorganisms and prebiotic compounds. In recent years, edible films and coatings additivated with prebiotic and probiotics have been the focus of much research, due to their potential in the edible food packaging field. The inclusion of probiotic microorganisms and prebiotics as bioactive compounds allows the development of edible functional food packaging. Understanding the characteristics of the different production methods of films and coatings allows the selection of those that are optimal for the bioactive compounds that are to be added. Working with probiotic microorganisms is complex since, as they are living bacteria, there are different chemical and physical factors that can reduce their viability. In this case, edible packaging can help to maintain an adequate concentration over time. The physical and mechanical characteristics of films and coatings depend directly on the interactions that take place between the biopolymers that make up their matrix. When probiotic bacteria and prebiotic compounds are added, these covalent and non-covalent interactions are modified, which leads to changes in the characteristics and properties of the edible packaging. There is still a profound need to understand the relationships between the different biopolymer interactions, especially when living bacteria are added to edible packaging, and also how the interactions influence the bacteriás stability within the matrix. Edible functional food packaging has great potential in the food industry, but more research is needed to better understand its behaviour in different foodstuffs and to allow the different food authorities to develop more precise rules and regulations for on its use in food products.

CRediT authorship contribution statement

S. Sáez-Orviz: Conceptualization, Investigation, Writing – original draft. **M. Rendueles:** Writing – review & editing, Supervision, Funding acquisition. **M. Díaz:** Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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