



Application of edible films containing probiotics in food products

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Abstract

The trend towards functional foods and healthy diets encourages researchers to produce new probiotic food products with improved probiotic delivery methods. Recently, edible films have been applied as carriers for probiotics, improving their survival in the foods and the gastrointestinal tract. This article is structured as follows: a description of edible films as probiotic carriers is followed by an introduction of the materials used as edible films in three main chapters (1) hydrocolloids (polysaccharides and proteins), (2) lipids and (3) composites. Then, probiotic viability in all mentioned edible films is discussed in detail and the impact of prebiotic incorporation is mentioned. Microbial, physicochemical, and sensory properties of edible film containing probiotics in food matrices are reviewed. Finally, the application of probiotic edible films in different food products is described.

Keywords Probiotic · Edible film · Viability · Functional food · Entrapment

1 Introduction

Probiotics can play a beneficial role in the human intestinal tract ecosystem (Abd El-Salam and El-Shibiny 2015). Today, increasing knowledge about the influence of diet on health leads to a continuous increase in the consumers' demand for functional food products, which exhibit minimum changes in organoleptic properties (Lusk 2019). This fact leads to an increasing attention of researchers to investigate certain characteristics of functional food products.

During industrial food processing and products' shelf life, probiotics are exposed to many challenges such as temperature, oxidative, acute toxic factors (e.g. hydrogen peroxide), water vapor, osmotic and mechanical stresses, and acid–base changes (Iaconelli et al. 2015). Also, probiotics

are adversely affected by low pH of the stomach, enzymatic action of pepsin, protease-rich conditions of the intestine, and contact with bile salts during their passage through the gastrointestinal tract (GIT) (Tripathi and Giri 2014). To provide health benefits, probiotics must be able to survive until their consumption and then successfully proliferate in the gut (Farnworth and Champagne 2010). The cell survival in probiotic food products is still challenging (Haffner et al. 2016; Maciel et al. 2014). Anhydrobiotics technology i.e. the encapsulation of viable cells via extrusion, spray-drying, emulsion, coacervation, and electrospraying, is the most popular method to maintain maximum viability of probiotics (Foroutan et al. 2017; Prasanna and Charalampopoulos 2018). One of the newest techniques to improve the survivability of probiotics is to use edible films, that are plasticized thin layered biopolymer structures and that protect them, besides having the potential to favor consumer health (Gialamas et al. 2010; Kanmani and Lim 2013; López de Lacey et al. 2012, 2014; Romano et al. 2014; Soukoulis et al. 2016).

It is possible to improve the functionalities of food products with edible films, such as incorporating nutrients, antioxidants, and antimicrobial agents. On the other hand, most of the edible films include high amounts of fiber, which are recommended in a healthy diet to weight control. In addition, probiotics can be incorporated into edible polymer matrices (via spray, freeze or fluidized bed drying) to

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improve food stability, functionality, and safety. Probiotic edible films are easier to prepare than encapsulated probiotics and are produced at lower cost because of lower equipment requirements (Altamirano-Fortoul et al. 2012). The application of edible films in the food industry can affect shelf life by reducing harmful reactions (Falguera et al. 2011). Furthermore, probiotic edible films can avoid gas exchange, moisture loss, photodegradation, oxidation, and growth of pathogenic microorganisms (Altamirano-Fortoul et al. 2012). Moreover, probiotic edible films could improve probiotic survival rates during storage and consumption and control probiotic dosage (Soukoulis et al. 2016).

The terms edible films and edible coatings represent different concepts. Edible films are thin solid layers that can be applied as a wrapping for foodstuff, while the edible coatings are formed directly on the surface of the food products (such as fruits), usually by immersing the products in a solution of structural matrix-forming materials (Silva-Weiss et al. 2013). Edible films can be applied to all kinds of food products like a cover, but usually, they are used for foods that cannot be immersed in the film-forming solution such as bakery products. However, probiotic edible films could not be a replacement for common packaging materials because these films are eaten with foods; therefore the products need an external packaging (such as paper packaging) to prevent external contamination. The difference between the probiotic edible coating and the probiotic edible film is demonstrated in Fig. 1. Since edible films are eaten with the products, nutritional and organoleptic characteristics of edible films

can be improved by inserting several components such as organic acids, essential oils, and chitosan (Rojas-Graü et al. 2009).

In recent years, probiotic immobilization in edible films has become considerably valuable (Guimaraes et al. 2018; Kanmani and Lim 2013; López de Lacey et al. 2014; Romano et al. 2014; Soukoulis et al. 2016). There are a few review articles about probiotic edible films (Espitia et al. 2016; Guimaraes et al. 2018; Pandhi et al. 2019; Pavli et al. 2018), but this study is a comprehensive review about probiotic edible films in foodstuff. It highlights the scientific investigations about probiotic incorporation in edible films and summarizes the materials used for edible films and probiotic applications in edible food packaging. In addition, the physicochemical and organoleptic properties of probiotic-containing edible films and probiotic viability in edible films are discussed. For this purpose, all recent and important published studies are reviewed comprehensively.

2 General aspects of probiotics: importance and application

Probiotics are defined as “viable microorganisms that, while ingested in sufficient amounts, exert health benefits on the host” (Huq et al. 2013). The main properties of probiotic strains are resistance to gastrointestinal conditions, adhesion to human epithelial cells or mucus, antimicrobial effects against pathogenic bacteria via the production of

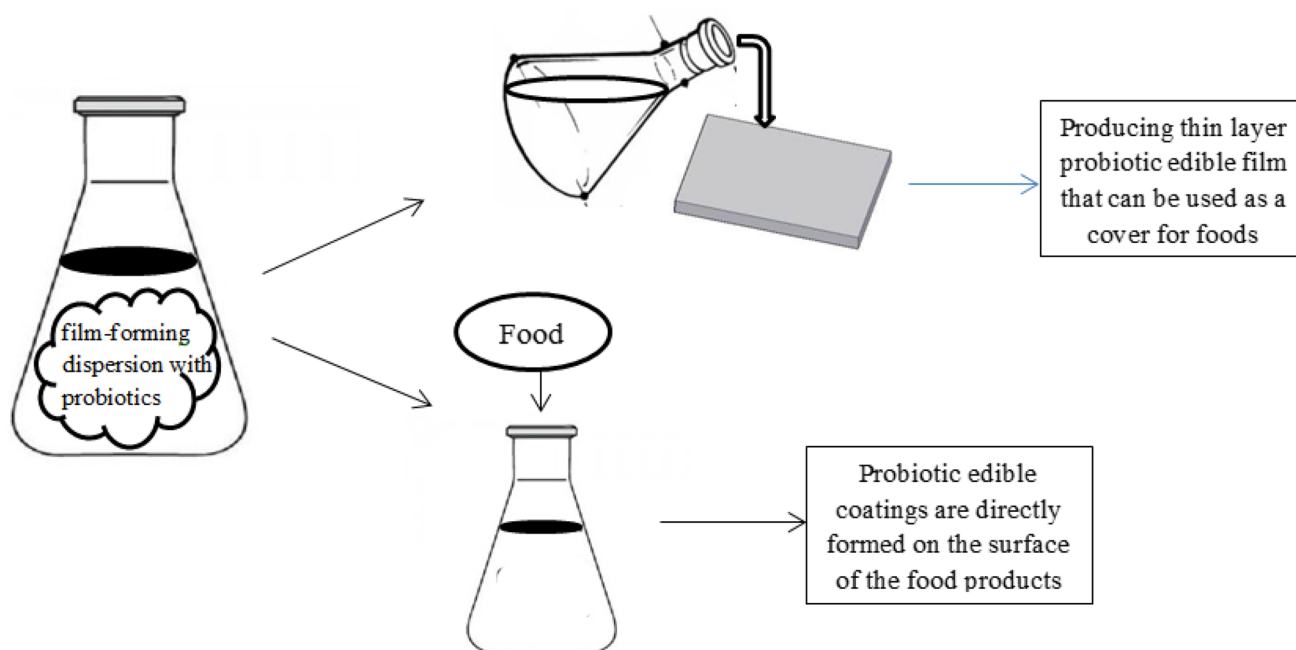


Fig. 1 The difference between the probiotic edible coating and probiotic edible film in foodstuff

antimicrobial agents or competition for growth factors or nutrients and binding sites (FAO/WHO 2002). Some of the reported health effects include improvement of GIT health (Thushara et al. 2016), reduction of serum cholesterol levels (Choi and Chang 2015), and biosorption of toxins (Zoghi et al. 2014, 2017) and heavy metals (Hadiani et al. 2018a, b) from foodstuff. To get the beneficial effects of probiotics, a daily intake of 10^7 – 10^9 Colony Forming Units (CFU)/g of probiotic viable cells per person for humans is recommended (Haffner et al. 2016).

3 Edible films as probiotic carriers

Although edible films are not a replacement for an external packaging, they enhance food protection by reducing the moisture loss and gas transfer between food and environment (Soukoulis et al. 2014a, b). Nowadays, edible films are applied as a carrier as well as a controlled release system for some drugs, antioxidants, antimicrobial agents. Since edible films act as water and oxygen barriers, enhancement of quality and shelf life of food products is the main result. Edible films are a “green” replacement for petroleum-based films and can be consumed with the food products (Soukoulis et al. 2014a, b). Immobilization of viable cells in biopolymer networks is a well-known technique to enhance microbial stability. Edible films have appropriate chemical and physical properties, and due to their sustainable nature they are proposed as bioactive compounds carriers (Falguera et al. 2011).

Edible films have the potential to create bespoke structures to enhance mechanical properties, increase shelf-life, and maintain structural integrity. Furthermore, they could be versatile and feasible carriers for the delivery of probiotics (Soukoulis et al. 2014b).

4 Incorporation of probiotics in edible films

Two major processes provide edible films:

1. In the wet process, biopolymers (such as methylcellulose) and other additives (such as plasticizers) are dissolved in distilled water, the film-forming solution is provided after homogenization, and then the solvent is evaporated.
2. The dry process is based on the thermoplastic behavior that some proteins and polysaccharides show at low moisture levels in pressing molding and extrusion (Guimaraes et al. 2018).

Tapia et al. (2007) first investigated the entrapment of probiotics into edible films. Since then, several studies

have evaluated the probiotic incorporation in edible films via the direct method, where the probiotic cells are added into the film-forming solution and the probiotic edible film is obtained by a casting method in a forced-air oven, and allowed to dry at room temperature (Kanmani and Lim 2013). Table 1 summarizes previous studies about edible films that have been used for probiotic incorporation in food products. Different parameters such as the presence of oxygen scavenging agents, the biopolymer and plasticizer type and amount, and adding prebiotics have recently been evaluated (Gialamas et al. 2010; López de Lacey et al. 2014; Romano et al. 2014; Soukoulis et al. 2013; Soukoulis et al. 2014b; Soukoulis et al. 2016).

5 Materials used for probiotic edible films

The materials used for the preparation of edible films can be categorized into three classes: hydrocolloids, lipids, and composites (Rojas-Graü et al. 2009). Some other materials such as plasticizers are also added to the film-forming solutions to enhance their stability or to improve the mechanical properties (Valencia-Chamorro et al. 2011).

5.1 Hydrocolloids

The hydrocolloids consist of proteins and polysaccharides. Proteins that are used for probiotic edible films include gelatin, wheat gluten, corn zein, soy protein, casein, and collagen. Protein-based probiotic edible films are prepared from protein solutions as the solvent (ethanol, water or their mixture) evaporates. It is reported that using protein could improve probiotic survival in edible films via scavenging free radicals and conveying nutrients (Burgain et al. 2013). In addition, applying proteins could lead to the formation of less porous and more compact structures (Soukoulis et al. 2016). Polysaccharides for probiotic edible films include cellulose derivatives, inulin, dextrans, alginate, starch derivatives, carrageenan, pectin derivatives, seaweed extracts, chitosan, and galactomannans. Polysaccharide-based probiotic edible films have good mechanical properties and form good odor, oxygen, and oil barriers, but their major disadvantage is their moisture permeability, due to their hydrophilic properties (Ramos et al. 2012). Protein edible films have better mechanical and barrier properties than polysaccharide edible films, but they present poor water resistance (Suput et al. 2015).

Alginates have good film-forming features and can provide crystalline and water-soluble edible films as probiotic carriers. Alginate is a common term for the alginic acid salts. Since alginate can eliminate lipid oxidation and delay dehydration, it has been applied mainly for meat products (Nayik et al. 2015).

Table 1 Probiotic edible films that have been investigated previously for probiotic incorporation in foodstuff

Probiotic	Additives	Edible film matrix	Food material	Storage condition	Probiotic viability ^a	Food shelf life	Physicochemical properties ^b	Sensory properties ^c	References
<i>L. sakei</i>	Sorbitol	Sodium caseinate	Fresh beef	4 °C and 25 °C	Increased viability	30 days	Physicochemical properties of the films were not significantly altered		Gialamas et al. (2010)
<i>Carnobacterium maltaromatum</i>	Nisin, glycerol	Alginate	Smoked salmon	4 °C	>7 log cycles CFU/cm ²	28 days			Concha-Meyer et al. (2011)
<i>L. acidophilus</i>	Carboxymethyl-cellulose, pectin, inulin and agave Carboxymethyl-cellulose	Starch	Bread		No significant reduction	24 h	Decrease in mechanical resistance, no differences in moisture content and water activity	No differences in taste or texture properties	Altamirano-Fortoul et al. (2012)
<i>L. acidophilus</i> , <i>B. bifidum</i>	Glucose (2%) and cysteine (0.05%) Sorbitol and glycerol	Gelatin	Chilled fish	2 ± 1 °C	Decreased 1 log CFU/g	10 days			López de Lacey et al. (2012)
<i>L. plantarum</i>	Glycerol	Sodium caseinate, pea protein, methylcellulose, hydroxyl propyl methyl cellulose	No substrate	5 °C and 75% RH	About 1 log CFU/cm ² reduction	30 days	Water vapor permeability increased. Elongation at break, tensile strength, and elastic modulus did not notably alter	No changes in transparency and color properties	Sánchez-González et al. (2013)
<i>L. rhamnosus</i> , <i>L. reuteri</i> , <i>L. acidophilus</i>		Pullulan, pullulan/starch blends	No substrate	4 °C	No significant reduction	30 days	Decreased viscosity, little effect on the water vapor permeability	Lower degree of transparency	Kanmani and Lim (2013)
<i>L. casei</i>	Inulin (0 to 4%), gelatin (2 to 5%)	Whey (8%), glycerol (6%), 5%	No substrate	25 °C	No significant reduction	10 days	Higher elasticity increased the viscosity		García-Argueta et al. (2013)

Table 1 (continued)

Probiotic	Additives	Edible film matrix	Food material	Storage condition	Probiotic viability ^a	Food shelf life	Physicochemical properties ^b	Sensory properties ^c	References
<i>L. rhammosus</i> GG	Whey protein concentrate	Sodium alginate	Pan bread	25 °C	> 10 ⁶ CFU/g up to 7 days	24 days	No major shifts in the physicochemical and thermal analysis physicochemical	Darker and higher red color intensity	Soukoulis et al. (2014b)
<i>L. rhammosus</i> GG	Polydextrose, inulin, glucose-oligosaccharides, wheat dextrin, glycerol	Gelatine	No substrate	Room and chilling temperature	33–80% reduction	63–100 days and 17–30 days at 4 and 25 °C	Increase of the matrix compactness and the reduction of porous and reticular structure detected		Soukoulis et al. (2014a)
<i>L. reuteri</i> , <i>L. acidophilus</i>	Glycerol	Methylcellulose, sodium caseinate	No substrate	5 °C	<i>L. acidophilus</i> > 10 ⁴ CFU/cm ²	30 days	Reduction in the elastic modulus and tensile strength at break		Sánchez-González et al. (2014)
<i>L. paracasei</i> L26, <i>B. lactis</i> B94	Green tea extract, glycerol, glucose	Agar	Hake fillets	4 °C	> 6 log cycles CFU/cm ²	15 days	Improve the physical properties (tensile strength and water vapor permeability)	No appreciable change of the color of the fish	López de Lacey et al. (2014)
<i>L. delbrueckii</i> , <i>L. plantarum</i>	Fructooligosaccharides	Methyl cellulose	No substrate	11–75% RH and 4 °C	1 log reduction	45 days 90 days	No significant physicochemical changes		Romano et al. (2014)
<i>L. plantarum</i> CIDCA 83114	Fructooligosaccharides	Methylcellulose	Green apple baked snacks	20 °C and 60% RH	Loss of 1.4 log CFU/g	90 days	No change in elastic modulus, increase in deformation	Taste, color, texture and overall acceptability did not alter	Tavera-Quiroz et al. (2015)
<i>L. rhammosus</i> GG	Sodium caseinate, soy protein concentrate, bovine skin gelatine caseinate	Corn and rice starch	No substrate	Fridge and room temperature conditions	0.91–1.07 log CFU/g decrease	27–96 days at fridge temperature and 15–24 days at room temperature	Film structures characterized by low porosity and high cohesiveness are associated with improved barrier and mechanical strength properties	No significant change in opacity	Soukoulis et al. (2016)

Table 1 (continued)

Probiotic	Additives	Edible film matrix	Food material	Storage condition	Probiotic viability ^a	Food shelf life	Physicochemical properties ^b	Sensory properties ^c	References
<i>B. animalis</i> and <i>L. casei</i>	Glycerol	Whey protein isolate	No substrate	4 °C and 23 °C	Reduction of 1–2 log cycles	60 days	No effects on films' thickness and mechanical properties	Color properties were maintained during storage period	Pereira et al. (2016)
<i>L. rhamnosus</i> GG	Whey protein concentrate	Sodium alginate, low esterified amidated pectin, kappa-carrageenan/locust bean gum, and gelatine	No substrate	4 °C and 25 °C	0–1.17 log CFU/g reduction	25 days	Soft, less fracturable and less rigid films, low tensile strength	Increasing both yellow and red hues and film opacity	Soukoulis et al. (2017)
<i>L. plantarum</i>		Pectin – starch	No substrate	Gastric condition	10 log → 1 log				Dafe et al. (2017)
<i>L. plantarum</i> , <i>L. pentosus</i>	Glycerol	Sodium alginate	Ham slices	4 °C, 8 °C, 12 °C	Reduced viability	66 days, 47 days, 40 days		The taste was more acidic than the control	Pavli et al. (2017)
<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i>	Glycerol	Carboxymethyl cellulose	No substrate	4 °C and 25 °C	decreased 3 log CFU	42 days	No significant physicochemical changes	Significant decrease in transparency	Ebrahimi et al. (2018)
<i>L. acidophilus</i> , <i>L. casei</i>	Glycerol	Sodium caseinate	No substrate	4 °C	> 4 Log CFU/cm ²	12 days	No significant effect on tensile strength	No significant effects on the optical properties	Abdollahzadeh et al. (2018)
<i>L. acidophilus</i>		Low acyl gellan gum and whey protein concentrate	No substrate	25 °C	A significant increase	30 days	The highest tensile strength	The lowest transparency value	González-Cuello et al. (2018)
<i>L. casei</i> TISTR 1463	Glycerol	Sodium alginate, gum arabic, and inulin	No substrate	4 °C	The highest microbial survival	100 days	The highest tensile strength and elongation property		Phovisay et al. (2018)
<i>L. rhamnosus</i> (GG)	Citric acid	Sodium carboxymethyl cellulose and hydroxyethyl cellulose	No substrate	25 °C			The mechanical, swelling and release properties can be tuned by HEC/CMC ratio and amount of cross-linker		Singh et al. (2018)
<i>L. acidophilus</i> LA3	Coconut water	Sodium alginate	Minimally processed carrots	8 °C without air circulation	Above 6 log CFU/g	21 days		Great acceptance	Shigematsu et al. (2019)

Table 1 (continued)

Probiotic	Additives	Edible film matrix	Food material	Storage condition	Probiotic viability ^a	Food shelf life	Physicochemical properties ^b	Sensory properties ^c	References
<i>L. casei</i>		Whey protein isolate	Cherry tomatoes and Thompson grapes	25 °C	5.70 to 7.77 log CFU/g solutes	28 days	No effect on the density and water vapor permeation, higher tensile strength, lower elongation at break		Dianin et al. (2019)
<i>B. animalis</i> subsp. lactis BB-12	Inulin	Whey protein isolate and alginate	Cereal bars	25 °C	No significant reduction	90 days	No significant effect on physico-chemical properties	Great acceptance	Pereira et al. (2019)

L. is Lactobacillus; B. is Bifidobacterium

^aProbiotic viability is evaluated after specific storage time compared to the initial number of probiotics (for each research)

^bPhysicochemical properties of edible film containing probiotic include mechanical properties, thickness, moisture content, and water vapor permeability

^cSensory properties for studies with food products are related to probiotic edible film with food; and for studies without food products are related to probiotic edible films themselves

Several investigations have studied pectin as a material for probiotic edible films (Espitia et al. 2016; Soukoulis et al. 2017). Pectins are structural constituents of plant cell walls and they are a common type of gelling agent.

Starch made edible films are usually tasteless, odorless, colorless, and transparent with low oxygen permeability. Starch edible films have good barrier properties to CO₂. Physicochemical and functional properties of starch-based probiotic edible films depend on the amylose/amylopectin ratio (Basiak et al. 2017). Additionally, cross-linked, oxidized, substituted, and acid-hydrolyzed starches are provided as a result of chemical modifications that have an impact on the edible film properties as probiotic carrier. Acid hydrolysis of starches decreases swelling power and increases solubility compared to native starches (Shah et al. 2016).

Gelatin-based probiotic edible films have poor water vapor barrier properties and are applied to meats, due to their ability in reducing oxygen, oil and moisture transportation (López de Lacey et al. 2012; Soukoulis et al. 2016). Gelatin has antimicrobial and antioxidant activities.

5.2 Lipids

Lipids used for probiotic edible films include vegetable oils, natural waxes, acetoglycerides, resins, and fatty acids. These compounds present some disadvantages such as chemical and mechanical instabilities and organoleptic quality reduction (Pavli et al. 2018). Therefore, lipids are commonly combined with other materials such as proteins or polysaccharides to improve specific characteristics of lipid-based edible films (Suput et al. 2015). Lipid-based edible films are good barriers to moisture transfer, but they have weak gas permeability and mechanical properties compared to polysaccharides and protein-based edible films (Guimaraes et al. 2018).

5.3 Composites

Composites are defined as probiotic edible films containing a blend of polysaccharides, proteins, and lipids. The purpose of providing composite edible films is to modify the properties of the edible film for specific applications such as carrying probiotics (Soukoulis et al. 2014a). A combination of materials can give more efficient properties to probiotic edible films because each material has its unique and limited functions (Rojas-Graü et al. 2009).

5.4 Plasticizers

Plasticizers are low molecular weight components, usually hydrophilic compounds, that decrease the glass transition temperature, increase toughness, flexibility and the tear resistance of the edible films. The plasticizers increase the intermolecular spacing, the mobility of polar polymer

chains, and decrease congested intermolecular forces and polymer chain gumminess. The most frequently used plasticizers include glycerol, sucrose, sorbitol, and polyethylene glycol (Ramos et al. 2012). Using plasticizers is essential for the formation of edible films, especially when polysaccharides or proteins are used as materials. However, it may increase the lethality of the embedded probiotic cells, due to osmolysis and increasing exposure to oxygen. To control this, some approaches including the incorporation of free radical scavenger compounds, promoting probiotic cell adhesion properties and suppression of matrix glass transition temperature are used (Burgain et al. 2013). Selecting a plasticizer for an edible film is based on the adaptability and persistence of the plasticizer, and the required physical characteristics of the films. Using sorbitol in the films leads to thermal stability, high thickness, and density. Applying glycerol increases moisture content. Moreover, edible films including glycerol show higher gas permeability in comparison to edible films including sorbitol (Pérez and Dufour 2017).

Rompothi et al. (2017) postulated that glycerol supplied better plasticizer efficiency than sorbitol. They also have concluded that enhancing plasticizer concentration led to increasing solubility, elongation, water vapor permeability, and seal strength, but decrease oxygen permeability, tensile strength, and elastic modulus. However, Krogars et al. (2003) have found that applying an equal amount of glycerol and sorbitol as a plasticizer was more effective than applying sorbitol or glycerol alone.

6 Probiotic viability in edible films

The edible films can maintain probiotic viability in the GIT (Soukoulis et al. 2014b). The survival of many probiotic strains have been widely evaluated under various conditions, whilst only a few investigations are accessible dealing with the viability of probiotics in edible films to evaluate their suitability as probiotic carriers (Guimaraes et al. 2018). Since the film-forming procedure and the chemistry of the edible films affect the probiotic viability (both post-processing and post-ingestion), they are critical factors (Soukoulis et al. 2017). While a complete mechanistic understanding of probiotic stability in edible film matrices during storage is not available, it is reported that steric hindrance of solutes and the interaction via hydrogen bonding with the polar head groups of membrane phospholipids, the presence of free radical scavenging agents and nutrients can be possible factors (Kanmani and Lim 2013; Soukoulis et al. 2013). Furthermore, the molecular mobility of solutes driven by the structural state of the immobilizing matrix can also affect the viability of probiotics. Therefore, obtaining low water matrices with low permeability to gases consisting of free radical

scavenging agents is an effective procedure for improving probiotics' survival in food products (Soukoulis et al. 2013).

The type of material used for probiotic-containing edible films might also cause considerable injuries because of osmotic stress (Bustos and Bórquez 2013). For instance, polysaccharides influence the viability of probiotics during the drying process and the storage period (Yonekura et al. 2013). In addition, the presence of high amounts of solutes, as well as the rubbery physical state (solute's increased molecular mobility) in the edible films, simplifies the occurrence of chemical and enzymatic reactions that injure cellular structures such as phospholipid membrane bilayers (Fu and Chen 2011).

The viability of *Lactobacillus (L.) casei* in sodium caseinate films with sorbitol as a plasticizer was studied by Gialamas et al. (2010). Direct entrapment of probiotics into the film-forming solution led to increased viability at both selected temperatures (4 °C and 25 °C). In addition, they stated that adding sorbitol increased viability due to its action as a protective agent for probiotics during drying or low water activity storage. The mechanism by which sorbitol and related polyols exert this protective impact could be due to the interaction between phospholipid membranes and sugars, helping to retain their fluidity (López de Lacey et al. 2012). Kanmani and Lim (2013) investigated probiotic viability in pullulan and starch-based edible films. *L. rhamnosus* GG, *L. reuteri* and *L. acidophilus* were entrapped into films of starches, pullulan, and their combination and were stored at 4 °C and 25 °C. Maximum probiotic viability was observed in pullulan films at 25 °C up to 30 days. Inserting starch to the pullulan films has a negative impact on cell viability. After 60 days of storage at 25 °C, no viable probiotic cells were observed, probably because of increased bacterial metabolism. In contrast, the pullulan and the pullulan-starch films maintained probiotic viability > 80% at 4 °C after 30 days, maybe because of decreased bacterial metabolism.

Concha-Meyer et al. (2011) incorporated lactic acid bacteria (LAB) into alginate films and used it to pack salmon. They reported that the LAB strain growth and viability in the edible film was improved after getting in touch with the salmon, maybe due to nutrient diffusion. They also claimed that the water activity of the edible film after drying ($a_w = 0.91$) affected probiotic viability because a low enhancement of this value ($a_w = 0.92$) was observed at the contact time with the salmon. Consequently, a decrease in water activity (or increase in osmotic stress) can impact the probiotic viability (Prasad et al. 2003). As stated previously, whey protein can partially decrease osmotic stress and increase adhesion that can lead to an improved survival rate (Soukoulis et al. 2017). Edible films without probiotics were applied as control samples in all studies.

Pavli et al. (2017) studied the viability of *L. plantarum* and *L. pentosus* in sodium alginate edible films. The storage

temperature had no impact on the survival of the probiotic cells. The viability was reduced after contact of edible films with ham slices at all temperatures, maybe because of the drying process and the subsequent stress. The viability of *Bifidobacterium* (*B.*) *animalis* Bb-12 and *L. casei*-01 in edible films were investigated by Pereira et al. (2016). They stated that whey protein had a positive impact on the viability of the probiotic strain during storage, due to its nutritional value and by increasing the buffering capacity.

In general, adding plasticizers into edible films enhances the molecular mobility of water and accelerates fatal enzymatic and chemical reactions. Low molecular mobility is obtained at low storage temperature and low water content (Tymczyszyn et al. 2012). Moreover, the permeability of the film to gases such as water vapor and oxygen can also affect adversely the viability of probiotic cells. Edible film composition (protein and polysaccharides type, type and amount of plasticizers, presence of prebiotics) and storage temperature play a key role in probiotics stability (Kanmani and Lim 2013; Soukoulis et al. 2014b).

6.1 Effect of incorporation of prebiotics

Prebiotics are mostly consisting of nonstarch polysaccharides and oligosaccharides that beneficially influence the host by selectively stimulating the activity or growth of one or a limited number of probiotic bacterial species in the colon, and therefore enhance their survivability for host health (Gallego and Salminen 2016). Although inserting prebiotics is a favorable strategy for probiotic protection, only a few researchers have investigated the use of prebiotics in probiotic-containing edible films to maintain stability and functionality of the entrapped probiotics.

The interaction of phosphate groups of lipid membranes with fructooligosaccharides reduces the phase transition temperatures. In addition, the dynamic mechanical analysis showed that the addition of fructooligosaccharides into edible films changes the hydrogen bonds in polymer molecules, decreases the glass transition temperature, thus indicating the plasticizer effect of fructooligosaccharides.

Therefore, the incorporation of fructooligosaccharides into the probiotic-containing edible films contributes to protecting probiotics during dehydration and storage (Romano et al. 2014). Moreover, inserting prebiotics into gelatin edible films led to a uniform and more compact structure, with no detectable interspaces, thus prebiotics could act as fillers in gelatin-based edible films (Soukoulis et al. 2014a).

7 Inhibitory effects of probiotic edible films against other microbial species

In addition to probiotic delivery to the consumers, probiotic edible films are important for increasing food stability and safety as the probiotics control the growth of spoilage microorganisms via competition or produce antimicrobial substances. It is well established that LAB show antimicrobial activity by the production of antifungal peptides, acids, and bacteriocins. Therefore, the incorporation of LAB into edible films also exhibits antimicrobial features (Cizeikiene et al. 2013) and reinsures the microbiological safety of the food products (García-Argueta et al. 2013).

Gialamas et al. (2010) stated that edible films containing *L. sakei* caused a significant reduction of *Listeria monocytogenes* in beef in comparison to the control. The inhibitory effect of *L. sakei* against *Listeria monocytogenes* has been related to direct competition for nutrients or to lactic acid production. The application of edible films containing LAB for chilled fish inhibited the growth of *Photobacterium phosphoreum* (López de Lacey et al. 2012). Sánchez-González et al. (2013) evaluated the inhibitory effect of *L. plantarum* incorporated in different edible films against *Listeria innocua*. They stated that polysaccharide-based edible films had significant activity against *Listeria innocua*, while protein-based edible films did not show this activity, may be due to the delay in bacteriocin production. They concluded that the material of the edible film and the time of bacteriocin production are the major parameters. Sánchez-González et al. (2014) confirmed the inhibitory impact of *L. reuteri* and *L. acidophilus* incorporated in sodium caseinate and methylcellulose against *Listeria innocua* for a week. Both film formulations have led to a decrease of approximately 1.5 log cycles of *Listeria innocua* growth at the end of storage. Thus, it can be concluded that the inhibitory activity is attributed to bacteriocin production, not to competitive growth, and the nature of the edible film matrix influences the bacteriocin production. In another study, *L. paracasei* and *B. lactis* embedded in the agar-based edible film into hake fillets caused a reduction of H₂S-producing microorganism counts during the storage period (López de Lacey et al. 2014). Edible films without incorporating probiotics were applied as control samples in all studies. Thus, probiotic edible films could be used as an alternative method of food preservation.

8 Probiotic-containing edible film characterization

8.1 Physicochemical properties

The physical and chemical properties of edible films should be analyzed simultaneously for the design of appropriate edible films with suitable plasticization properties providing a suitable environment that guarantees the viability of the probiotic.

8.2 Mechanical properties

Edible films, as probiotic carriers, should have appropriate mechanical strength and extensibility to resist food processing, packaging, and storage stress (Garavand et al. 2017). The presence of structural deficiencies, the structural adaptation of the major components of edible films, the distribution and density of the intra- and intermolecular interactions between the polymer chains, and the type and amount of plasticizers have been reported to affect the mechanical profile of edible films as probiotic carriers (Falguera et al. 2011).

The plasticizer glycerol is an effective parameter that determines the mechanical properties of edible films as probiotic carriers. It reduces the intermolecular forces between polymers, reducing the tensile strength and increasing the elongation at break (Rouhi et al. 2017). Moreover, the presence of cavities and holes could decrease the edible film flexibility and tensile strength. Therefore, edible films must have good mechanical properties to protect probiotics, resist food processing, handling, and storage stresses. Gialamas et al. (2010) demonstrated that no significant changes were observed in tensile strength, elongation at break, and modulus of elasticity of sodium caseinate edible films containing probiotics, because of the relatively low mass of the added probiotics with little impact on the mechanical properties of protein-based edible films. Consequently, the cellulose-based edible films, with greater mechanical resistance, are a little more sensitive to the incorporation of the probiotic cells.

8.3 Thickness

Thickness is a crucial parameter that affects the water vapor permeability, transparency, and mechanical features of the edible films to improve the film's ability to carry probiotics and to increase the mechanical integrity of foods (Ghanbarzadeh and Almasi 2011). The thickness of edible films depends on the preparation technique and drying conditions (Galus and Lenart 2012). Soukoulis et al. (2014a) stated that

no significant impact on thickness was observed by the addition of *L. rhamnosus* GG cells into the probiotic-containing edible film. Conversely, Soukoulis et al. (2014b) in another study demonstrated that the inclusion of probiotics in film-forming solutions changed the film thickness.

8.4 Moisture content

Since edible films consist of hygroscopic materials, and a high water content is harmful for probiotic preservation, the control of the moisture content during food processing and storage is important (Soukoulis et al. 2014b). The moisture content after drying influences the rate of probiotic viability during long storage periods and simplifies the melting of edible films in the mouth (Kanmani and Lim 2013). Glycerol can retain the water content in the edible films and thus prevent water evaporation (Thakhiew et al. 2010).

8.5 Water vapor permeability

Water vapor permeability is one of the most critical properties of edible films as probiotic carriers, that can be influenced by parameters such as solubility coefficient, diffusion rate, hydrophobic ratio, the integrity of the film, interactions between the functional groups of the polymers, crystalline ratio, thickness, and amorphous ratio (Kanmani and Lim 2013). Water vapor permeability of the edible films is influenced by the mobility of polymer chains (Su et al. 2010). The probiotic cells, as discontinuous particles, might inhibit the chain mobility of the polymers in the film matrix (Guimaraes et al. 2018). Moreover, increased water vapor permeability may improve edible film solubility, which is one of the main advantages to releasing probiotics (Kanmani and Lim 2013).

8.6 Sensory properties

Acceptable sensory properties of probiotic products are required for achieving commercial success in the market. Therefore, sensory assessment is essential before starting a new production. It is well established that inserting free probiotics into food products can significantly alter their sensory properties. Entrapment of probiotics into edible films can control undesirable modifications in sensory properties (Corona-Hernandez et al. 2013). Although there are some studies regarding the sensory properties of edible coating containing probiotics, there is a gap of knowledge dealing with edible films containing probiotics.

The color and optical properties of edible films are important because they directly affect the appearance of food products and consumers' preferences. Transparency is one of the usual optical properties of edible films. Inserting probiotics into the edible films may influence the light that passes through

the edible film, perhaps because of the increasing dispersion of light (Kanmani and Lim 2013). According to Martins et al. (2012), the moisture content of probiotic-containing edible films can affect the light reflections on the film surface. Moreover, Ghanbarzadeh and Almasi (2011) observed an improvement in optical properties and yellowness reduction by inserting carboxymethyl cellulose to starch-based films. The shine of the edible films depends on the surface morphology achieved during film drying. Generally, the smoother the surface of the edible films, the higher the brightness (Ward and Nussinovitch 1996).

Due to the difference between the density of cells and the polymer solution during the drying stage, probiotics are present on the surface of the edible films and form a continuous layer. The number of probiotic cells accumulated on the edible film surface depends on the viscosity of the film-forming dispersion (Ly et al. 2008). The surface charge of the probiotics is essential because of the determination of electrostatic interactions with charged polymers. Generally, parameters such as the crystallites' mean size and crystallinity, the amount of plasticizer and its type as well as structural conformation, the refractive index, and compatibility of the film compounds affect the opacity of edible films (Fakhouri et al. 2013).

Tavera-Quiroz et al. (2015), applied edible films of methylcellulose containing *L. plantarum* to green apple baked snacks. The analyzed sensory attributes included taste, color, texture, appearance, and overall acceptability. The snacks' taste score rating with the probiotic-containing edible film was considerably lower than that of the control without the probiotic edible film. In addition, the color-related findings were nearly identical, but the texture values of control samples were slightly higher than that of the snacks with probiotic edible film, perhaps because of their higher moisture content. Similarly, Alvarez (2012) stated that edible film with a specified composition (milk whey, inulin, and glycerol) can maintain the textural properties of the broccoli during 15 days of storage. Sodium alginate edible films containing probiotics significantly influenced the aroma and taste attributes of ham slices and the total organoleptic scores. The researchers demonstrated that these disadvantages were partially controlled when a high-pressure processing treatment was applied before using the probiotic-containing edible films, probably due to a lower level of probiotic cells number in the ham slices (Pavli et al. 2017).

9 Application of probiotic edible films in food products

Compared to conventional dehydrated microcarriers, probiotic edible films could provide significant benefits, e.g. increasing the shelf life period, for intermediate moisture foods, because

of their ability to maintain their physical state and biological activity during food storage. Therefore, several applications for probiotic edible films such as fruits and vegetables, bakery and confectionery products, olives, dairy products, fishery products, cereal bars, and meat products have been studied (Altamirano-Fortoul et al. 2012; De Prisco and Mauriello 2016; Espitia et al. 2016; López de Lacey et al. 2012; Soukoulis et al. 2017; Tavera-Quiroz et al. 2015).

The first application of probiotic edible films was evaluated for fresh fruits (Tapia et al. 2007). After that, probiotic edible films were applied to carrots (Shigematsu et al. 2019), cherry tomatoes, and Thompson grapes (Dianin et al. 2019). Probiotic edible films for fresh fruits and vegetables could control ripeness by decreasing oxygen diffusion and inhibiting the growth of spoilage microorganisms, so that metabolic activity and softening changes will be reduced. Therefore, the freshness of the fruits, the quality characteristics such as color, acid, sugar and flavor and the nutritional factors can be maintained for a longer time (Pandhi et al. 2019).

Although there are some investigations (Concha-Meyer et al. 2011; Gialamas et al. 2010; López de Lacey et al. 2012, 2014) about using probiotic edible films in meat and fishery products, further studies are needed. The efficiency of sodium-alginate edible films as the probiotic carrier, was evaluated for application in sliced ham with or without high-pressure processing pretreatment. The results of the study demonstrated that probiotic strains were successfully carried by edible films in the meat products irrespective of the high-pressure processing treatment (Pavli et al. 2017). In another study, *L. acidophilus* and *B. bifidum* were inoculated into edible gelatin films for preserving hake fish (*Merluccius merluccius*) (López de Lacey et al. 2012). Adding green tea extract into probiotic edible films (containing *L. paracasei* and *B. lactis*) resulted in better chemical and microbial stabilities and prolonged the shelf-life of hake fillets for at least one week (López de Lacey et al. 2014).

Some researchers focused on the effect of the application of probiotic edible films on the shelf-life and quality factors in bakery and confectionery products (Altamirano-Fortoul et al. 2012; Soukoulis et al. 2014b, 2017; Tavera-Quiroz et al. 2015). The outward appearance of baked products is the main quality factor that influences the visual sense of the consumers. Soukoulis et al. (2017) found that no significant difference was observed in sensory and thermo-physical characteristics of bread samples in bread conventional packages and bread with the probiotic edible film.

10 Conclusions

In this article, the probiotic incorporation into edible films to improve probiotic viability during the storage period and processing of the food product has been reviewed.

Application of edible films as a carrier of probiotics has several benefits. Indeed, many of these films contain high amounts of fiber, which are recommended in a healthy diet. Fibers can also stimulate satiation signals in the colon which may be useful for weight control.

A wide variety of edible film materials with different properties can be applied which may influence the properties of the final products. Although the physicochemical properties of edible films can be influenced by the incorporation of probiotics, using edible films containing probiotics does not alter the sensory characteristics of the food products significantly. In general, the application of the edible film is a promising strategy to enhance probiotics' survival during storage time.

Although the incorporation of probiotic bacteria into many different edible materials is reported and survival and properties of films have been studied, there is a lack of in vivo studies and investigation of survival and colonization properties of such entrapped probiotics in the human body. In addition, there is no report on the inhibitory effects of probiotic films against pathogens in vivo. What is still mystifying scientists in the field of probiotic-containing edible films, is that how an entrapped microorganism can be released from the film, activated and localized during the short time of passage (on average a few hours) in the GIT.

Moreover, many technological and economic aspects of manufacturing processes need to be streamlined. Further studies must be carried out to apply different probiotics and prebiotics in new edible films and apply them to foods familiar for the consumer. Each geographic region should provide edible films with their localized and accessible probiotics, prebiotics and film materials to apply in their localized foods to increase the efficacy and health benefits of functional foods.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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