Chapter 2
Edible Films

María R. Ansorena, Mariana Pereda, and Norma E. Marcovich

Abstract Self-supporting edible films are one of the emerging technologies used today to optimize food preservation. With an ever increasing demand of consumers for high quality food products in addition to environmental concerns regarding the adverse effect of plastic packaging, food industry drive to develop and implement new types of edible films. The use of edible films as food packaging can play an important role in the quality, safety, transportation, storage, and display of a wide range of fresh and processed foods. Edible films can provide replacement and/or fortification of natural layers preventing moisture losses, while selectively allowing for controlled exchange of important gases such as oxygen and carbon dioxide, therefore, extending shelf life by minimizing food quality deterioration. Moreover, edible films can act as carriers of food additives such as vitamins, antimicrobial and antioxidants agents, providing a highly localized functional effect and improving food organoleptic properties. In addition, edible films could add value to agricultural and food industries by-products, since they are formed from various renewable eco-friendly and edible substances such as proteins, lipids or carbohydrates. Lipid-based films have good water barrier properties but form brittle films. On the other hand, protein and polysaccharide based films generally have good mechanical properties and thus they may withstand handling. However, they are not good barriers to water vapor. The use of blends comprising such compounds or their combination with lipids is thus a way of developing composite edible films matching the requirements for use as food packaging. Accordingly, this chapter discusses the latest advances on edible films aimed for food packaging.

Keywords Active films · Bioactive films · Food packaging · Food preservation

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2.1 Introduction

Changes in the current lifestyles of modern consumers have led to an increase in the sales of ready-to-eat (RTE), minimally processed and preservative-free food products (Peelman et al. 2013; Raybaudi-Massilia et al. 2016; Nisar et al. 2017; Moghimi et al. 2017). Consumers are demanding microbiologically safe and fresh-like products that are healthy, shelf-stable, convenient, and produced using environmentally friendly technologies (Raybaudi-Massilia et al. 2016). Nevertheless, natural and preservative-free food products are more susceptible to be attacked by spoilage and pathogenic microorganisms due to their manipulation. The growth of spoilage microorganisms and food-borne pathogens is one of the most important causes for food degradation (Viuda-Martos et al. 2011; Atarés and Chiralt 2016; Giteru et al. 2017), since it can accelerate lipid and other oxidation processes, produce changes in the organoleptic properties of the foods (Saggiorato et al. 2012; Atarés and Chiralt 2016) and losses of nutritional quality (Ganiari et al. 2017). Moreover, food-borne pathogens are directly responsible for certain illnesses in the human organism, or can be indirectly responsible due to the production of toxins (Saggiorato et al. 2012; Atarés and Chiralt 2016).

Packaging materials play an important role in containing foods and preserving the quality and safety of a food product throughout the supply chain until consumption (Raybaudi-Massilia et al. 2016; López-Córdoba et al. 2017). The packaging material functions as a barrier to the migration of pollutants from the environment to the product (Raybaudi-Massilia et al. 2016). It is however also urged to meet aesthetic and mechanical requirements for food applications (Manrich et al. 2017). For more than 50 years, the food industry has come to use a wide range of synthetic petroleum-based polymers that have provided convenience and ease of use in different situations (Giteru et al. 2017). However, questions have been raised about their continued use: they are non-renewable and non-biodegradable and therefore they have become a serious environmental issue that concerns all the stakeholders of the food production chain (Raybaudi-Massilia et al. 2016; Vijayendra and Shamala 2014; Giteru et al. 2017; López-Córdoba et al. 2017). Therefore, innovative films derived from agro-food industry wastes and renewable low cost natural resources have received greater attention as effective and economical replacement for conventional plastics (Valdés et al. 2014; Balti et al. 2017; Nisar et al. 2017; Alzate et al. 2017). Some of these materials are edible, so they can be consumed together with the product or be in close contact with the food, while being eco-friendly and ensuring that the package meets the primary objective of protecting the food (Raybaudi-Massilia et al. 2016).

Edible films are preformed, thin layers, made of edible materials, which once formed can be placed on or between food components (McHugh 2000; Yuan et al. 2016). Besides being biodegradable and compostable, edible films are desirable because they also offer a lucrative outlet for surplus agricultural materials (Tomasula 2009). On the other hand, these films have similar functions as those of conventional packaging, including barriers against water vapor, gases, and flavor

compounds and improving structural integrity and mechanical-handling properties of foods (Galus and Kadzińska 2015; Yuan et al. 2016). Smartly designed, they can also improve the quality, safety, shelf life, and functionality of food products, as well as increase food sensory attributes and convenience (Pascall and Lin 2013; Raybaudi-Massilia et al. 2016), while minimizing both spoilage and pathogenic microorganisms during storage, transportation, and handling (Arrieta et al. 2014; Raybaudi-Massilia et al. 2016; Gutierrez-Pacheco et al. 2016). Moreover, the edible films can be used to produce a soluble package for premixed food ingredients or additives and act as a separate layer of individual food portion (Harnkarnsujarit 2017).

2.2 Materials Used for Edible Films

Edible films can be made from materials such as lipids, proteins, and polysaccharides with the ability to form films (Gutierrez-Pacheco et al. 2016; Balti et al. 2017; Hashemi and Mousavi Khaneghah 2017; Ganiari et al. 2017; Dehghani et al. 2018). The polysaccharides used for making edible films include cellulose derivatives, chitosan, starch, starch hydrolysates (dextrins), konjac flour, gums, pullulan, alginate, carrageenan, pectin and others that should be chemically treated to increase water solubility like cellulose and chitin (Park et al. 2014; Desobry and Arab-Tehrany 2014; Dehghani et al. 2018; Ganiari et al. 2017). Polysaccharides are widely available and usually cost effective (Dehghani et al. 2018). Due to the presence of a large number of hydroxyl and other polar groups in their structure, hydrogen bonds have a crucial function in film formation and final characteristics (Dehghani et al. 2018; Harnkarnsujarit 2017). The major mechanism of film formation in polysaccharide films is the breaking apart of polymer segments and reforming of the polymer chain into a film matrix or gel. This is usually achieved by evaporation of a solvent creating hydrophilic and hydrogen bonding and/or electrolytic and ionic cross-linking (Park et al. 2014). Protein films originate from several sources including plant, meat, egg, and milk, for example, collagen, albumin, gelatin, casein, milk whey proteins, corn zein, ovalbumin, soy protein, peanut protein, pea protein, rice bran protein, cottonseed protein, keratin and wheat gluten (Harnkarnsujarit 2017; Kumari et al. 2017; Park et al. 2014; Desobry and Arab-Tehrany 2014; Ansorena et al. 2016). However, some considerations with respect to food intolerances, such as wheat gluten intolerance (celiac disease), or milk protein intolerance, allergies, or religious beliefs/banning, should be taken into account when protein-based films and coatings are used (Desobry and Arab-Tehrany 2014). The main mechanism of formation of protein films includes denaturation of the protein initiated by heat, solvents, or a change in pH, followed by association of peptide chains through new intermolecular interactions (Dehghani et al. 2018), being the protein-protein interactions, with disulfide, hydrogen, and hydrophobic bonds, the main associative forces in the film network (Park et al. 2014). Proteins have good film-forming properties and good adherence to hydrophilic surfaces (Dehghani et al. 2018).

Protein and polysaccharide-based films generally have good mechanical and sensory properties and are effective barriers to aroma compounds and gases such as oxygen and carbon dioxide, but due to their hydrophilic nature, they are not effective water vapor barriers (Desobry and Arab-Tehrany 2014; Otoni et al. 2016). Even so, protein or polysaccharide based films can be used as "sacrificing agents" that retard moisture loss from the food products by adding additional moisture on the surface that is lost first, as indicated by Kester and Fennema (1986). Contrastingly, lipid-based films have good water barrier properties due to their apolar nature, but possess undesirable sensory properties and form brittle or non-cohesive films (Desobry and Arab-Tehrany 2014; Otoni et al. 2016). Therefore, lipids are either used as coatings or incorporated into polysaccharides or proteins matrices to form composite films, giving a better water vapor barrier, due to their low polarity (Desobry and Arab-Tehrany 2014; Dehghani et al. 2018). Lipid incorporation into edible films and coatings can improve also cohesiveness and flexibility making better moisture barriers (Desobry and Arab-Tehrany 2014). Some of the lipids that have been used effectively in films or coating formulations are beeswax, mineral oil, vegetable oil, surfactants, acetylated monoglycerides, shellac, terpene, carnauba wax, and paraffin wax (Desobry and Arab-Tehrany 2014; Harnkarnsujarit 2017). Lipids offer limited oxygen barrier properties, due to the presence of microscopic pores and elevated solubility and diffusivity (Desobry and Arab-Tehrany 2014) and are mostly soft solids at room temperature (Harnkarnsujarit 2017). Distribution of chemical groups, the length of the aliphatic chains, and the presence and degree of unsaturation impact lipid polarity and thus, they could behave in different ways respect to moisture transfer. For example, waxes (esters of long-chain aliphatic acids with long-chain aliphatic alcohols) have very low content of polar groups and high content of long-chain fatty alcohols and alkanes and thus they are very resistant to water migration while the high polarity of the unsaturated fatty acids results in high moisture transfer (Dehghani et al. 2018).

Whichever material is used for films, it should form an unbroken film structure with good functional properties (Kumari et al. 2017), i.e. food packaging having poor mechanical properties may not withstand handling, whereas one with poor barrier properties may lead to food physical, chemical, and microbiological spoilage (Otoni et al. 2016). To achieve this generally protein and polysaccharide-based film formulations require the addition of a plasticizing agent above a minimum threshold to reduce film fragility, confer certain plastic properties (Kokoszka et al. 2010; Martins et al. 2012; Sánchez-Ortega et al. 2014; Costa et al. 2015; Pérez et al. 2016) and sometimes also to guarantee their processability (Martins et al. 2012). The plasticizer molecules modify the three-dimensional organization of the polymeric materials, leading to decreased intermolecular forces along the polymer chains, increased free volume and chain mobility, thus improving flexibility, extensibility, and toughness of the film (Kokoszka et al. 2010; Pérez et al. 2016). However, plasticizers also decrease film cohesion and thus the mechanical resistance and barrier properties of the films (Pérez et al. 2016). The most commonly used food-grade plasticizers are sorbitol, glycerol, mannitol, sugar and polyethylene glycol (Kokoszka et al. 2010; Pérez et al. 2016; Castro-Rosas et al. 2016). On the other

hand, it was demonstrated that lipids sometimes can also plasticize edible films (Pereda et al. 2012; Rodrigues et al. 2014; Kowalczyk et al. 2016; Rocca-Smith et al. 2016; Otoni et al. 2016) possibly because the amphiphilic compounds (e.g. acetic acids of mono and di-glycerides, and glycerol monostereate) included in the lipid phase, could act as lubricants favoring sliding of molecular chains, leading therefore to a gentle and highly extensible polymer network, as pointed out by Rocca-Smith et al. (2016).

2.3 Composite Edible Films

As can be deduced from the previous section, one of the most used approach to tailor films' properties and thus to develop edible films matching the desired functional properties and requirements for use as food packaging, has been the obtaining of composite films based on the combination of different biopolymers, biopolymers and lipids, biopolymers and solid particles (i.e. nonsoluble substances such as fibers, hydrophobic proteins, organic, and/or inorganic nanoparticles (NPs)), and so on (Martins et al. 2012; Desobry and Arab-Tehrany 2014; Otoni et al. 2016; Balti et al. 2017; Hashemi and Mousavi Khaneghah 2017; Ganiari et al. 2017). As expected, the main objective is to take advantage of the properties of each compound but also to benefit from the synergy between them when possible (Ganiari et al. 2017). In this sense, composite films are those whose structure is heterogeneous, that is, composed of a continuous matrix with some added phases or composed of several layers (Buffo and Han 2005; Fortunati 2016; Ganiari et al. 2017). Usually, multilayered films have better mechanical and barrier efficiencies than emulsion-based films and coatings, but their manufacturing requires an additional step of spreading or lamination and drying for each layer that could lead to layer delamination (Otoni et al. 2016). Moreover, in an industrial plan they do not seem very practical because of too many steps in their manufacture. On the contrary, solution or emulsion-based edible films could provide nearly the same properties, while only requiring one operation in their preparation (Fortunati 2016). However, when dealing with film-forming emulsions usually the incorporation of an emulsifier to avoid phase separation during drying is required (Otoni et al. 2016), which adds complexity to the system. Furthermore, the mechanical and barrier properties of these films not only depend on the compounds used in the polymer matrix, but also on their compatibility (Ganiari et al. 2017). Nevertheless, there has been great scientific and commercial progress made in this area (Balti et al. 2017; Hashemi and Mousavi Khaneghah 2017) and thus, some recent examples of the obtained composite films are presented below. The following works were selected because their systems were able to exhibit synergistic contributions instead of a range of properties that go from those of one of the constituents to the other one, like the rule of mixtures could predict.

Kurt et al. (2017) optimized the concentrations of two polysaccharides, xanthan (XG) and locust bean gum (LBG), and glycerol as plasticizer in the film formulation using combined design (a statistical tool that consists of mixture design and response

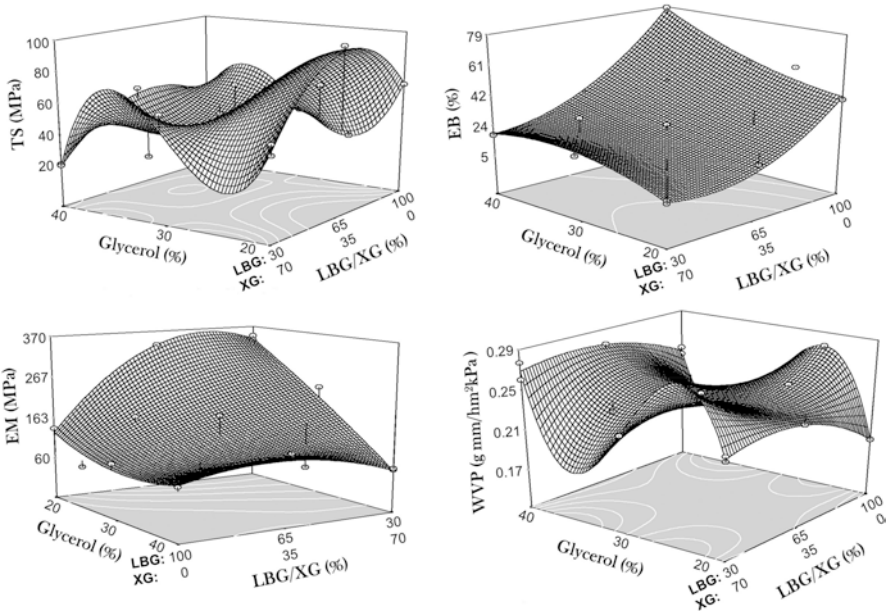

Fig. 2.1 Effect of different gum combinations and glycerol concentration on the mechanical and water vapor permeability properties of the edible films (*TS* tensile strength; *EB* percentage of elongation at break; *EM* elastic modulus; *WVP* water vapor permeability). From Kurt et al. (2017) with permission

surface methodology). The effect of all LBG/XG and glycerol concentrations used on the film's mechanical and water vapor permeability (WVP) properties is illustrated in Fig. 2.1. According to the authors, these two polymers had excellent blend miscibility and exhibited synergic interactions between them that allowed to maximize almost all the functional properties of the resulting edible film at 89.6%, 10.4% and 20% of LBG, XG and glycerol, respectively. At this optimum point, the WVP, tensile strength (TS), elongation at break (E%) and elasticity modulus (EM) values of film resulted 0.22 g mm h^{-1} m^2 kPa, 86.97 MPa, 33.34% and 177.25 MPa, respectively. Manrich et al. (2017) obtained water-resistant edible films derived from the tomato peel-extracted biopolyester cutin upon its casting in the presence of pectin (a water soluble polysaccharide). The obtained films were able to mimic tomato peel in terms of mechanical strength and thermal stability. Nevertheless, uniform surface hydrophobicity and greater stiffness were observed for cutin/pectin films as compared to that naturally occurring ultrastructure. Moreover, cutin-based edible films resulted slightly affected by moisture and thus, they were recommended to be used as water-resistant plastic wraps for short-time applications.

Silva et al. (2016) manufactured blended glycerol-plasticized films using whey protein isolate (WPI), four different concentrations of locust bean gum (LBG) and two different thermal treatments. Authors found that interactions between WPI and LBG strongly influence film properties and it is thus possible to tune the properties

of WPI-based edible films to meet food packaging and edible coating needs adding different amounts of LBG and/or using different thermal treatments. The composites resulted more stable as longer was the thermal treatment due to the higher cross-linking effect and higher level of developed molecular interactions. This, consequently, resulted in the obtaining of stronger and less soluble films with improved barrier properties to carbon dioxide, oxygen and light. For example, films made from solutions with 5% WPI + 0.1% LBG + 2% glycerol, heated at 75 °C for 10 min presented the lowest oxygen permeability (more than 50% decrease as compared with the film with no LBG, with the same thermal treatment).

Kowalczyk et al. (2016) studied the effect of adding different lipids on the physicochemical and morphological properties of sorbitol-plasticized pea protein isolate (PPI) edible emulsion films. The chosen lipids were anhydrous milk fat (AMF), candelilla wax (CNW), lecithin (LEC) and oleic acid (OLA) and were incorporated into film-forming solutions at 0, 0.5, 1.0, 1.5, and 2.0%. Authors found that only AMF and CNW reduced WVP of the films, this last one in a dose dependent manner, leading to a WVP 2.5 times lower than that of the control for the films incorporated with 2.0% CNW. On the other hand, the incorporation of lipids into PPI films caused an increase in oxygen permeability and decreased the mechanical strength. Nevertheless, all the produced films were effective UV barriers. Surface microstructure of the emulsion films was influenced by the lipid type and lipid volume fraction, as shown in Fig. 2.2. Unlike the solid lipids, OLA did not reduce the film transparency and showed a plasticizing effect, making the films more extensible. Summarizing, this work showed that CNW was the most effective lipid for this system since it was able to improve the water vapor barrier properties and simultaneously provoked the lowest increase in the oxygen permeability and the lowest decrease in the mechanical strength of the films. Besides, CNW-added films had also higher transparency compared to the other films containing solid lipids (AMF, LEC).

Hosseini et al. (2015) successfully developed bio-nanocomposite films based on fish gelatin (FG) and spherical chitosan nanoparticles (CSNPs) with size range 40–80 nm. The incorporation of CSNPs to FG films improved their water vapor barrier, as well as TS and elastic modulus, which was associated to the evenly dispersion of the particles in the bio-polymeric matrix at lower loading levels (less than 8%, w/w) added to the interaction between CSNPs and FG through hydrogen bonding. Furthermore, addition of CSNPs contributed to the significant decrease of WVP, leading to a 50% reduction at 6% (w/w) filler. Composite films presented reduced values of transparency at 600 nm as compared to the control film (0% CSNPs) while they have excellent barrier properties against UV light. The results presented in this study show the feasibility of using bionanocomposite technology to improve the properties of biopolymer films based on fish gelatin. Antoniou et al. (2015) produced composite tara gum films with the inclusion of both, bulk chitosan or chitosan nanoparticles at various concentrations. The incorporation of bulk or chitosan nanoparticles resulted in improved mechanical (the TS of the control film films was 22.71 ± 2.98 MPa and increased, respectively to ~58 MPa or ~53 when 10 or 15 wt% of CSNPs or bulk chitosan were added, without reducing notably the

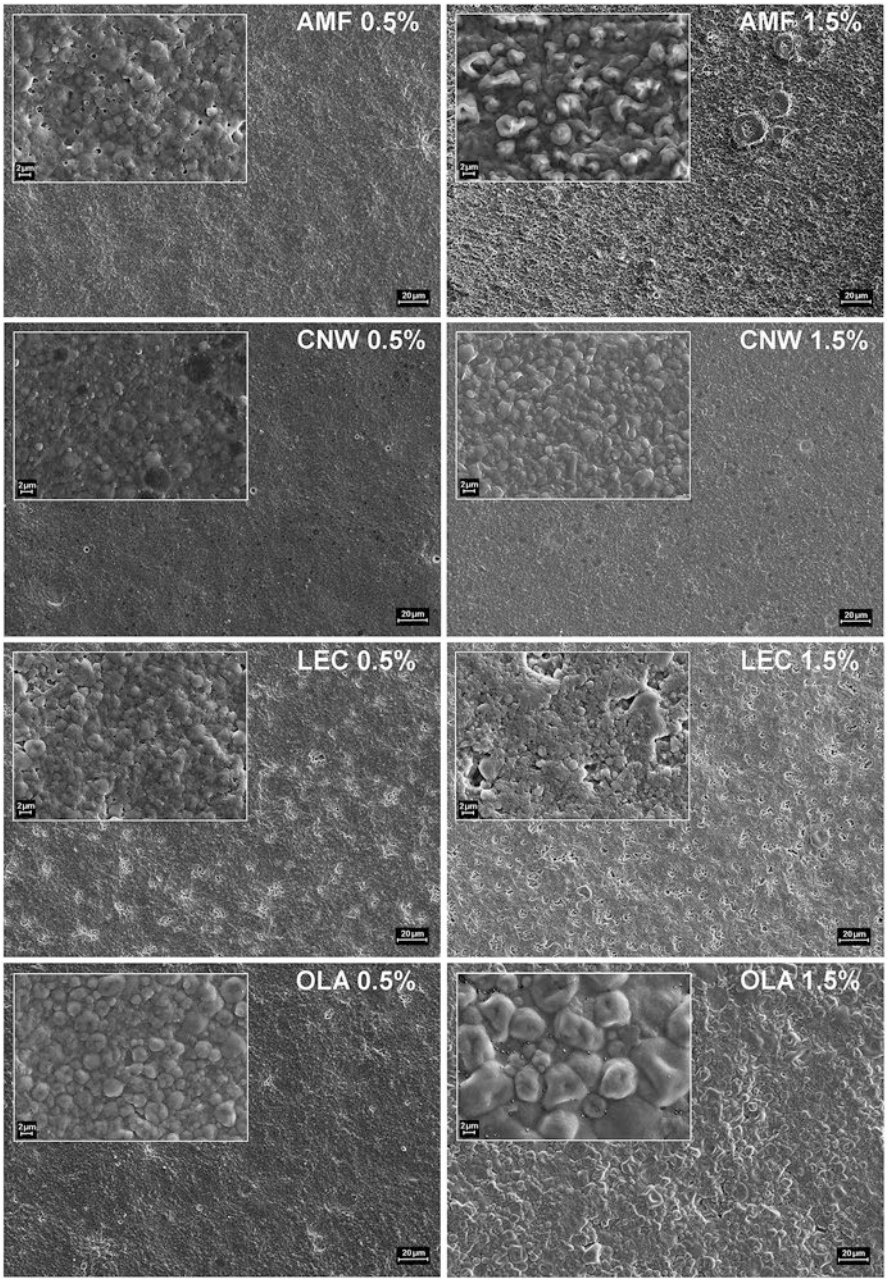

Fig. 2.2 SEM micrographs (×1000 and ×10 000 magnification) of surfaces of the emulsion PPI-based films. From Kowalczyk et al. (2016) with permission

Table 2.1 Antimicrobial activity of tara gum films containing bulk chitosan (CS) and chitosan nanoparticles (CSNPs) against food pathogenic bacteria of *E. coli* and *Staphylococcus aureus*[a]

Film type	CS/CSNP content (% w/w)	Inhibitory zone (mm^2)	
		E. coli	*S. aureus*
TG	0	0.0 ± 0.0^{f}	0.0 ± 0.0^{d}
TG + CS	5	65.43 ± 10.36^{d}	78.45 ± 10.13^{c}
	10	112.0 ± 16.06^{b}	117.96 ± 11.08^{b}
	15	138.87 ± 12.35^{a}	157.42 ± 10.95^{a}
TG + CSNPs	5	41.53 ± 7.60^{e}	85.30 ± 12.30c
	10	87.32 ± 6.72^{c}	111.71 ± 3.12^{b}
	15	85.80 ± 6.17^{c}	93.41 ± 8.59^{c}

From Antoniou et al. (2015), with permission

[a]Values are given as mean ± standard deviation. Different superscript letters in the same column indicate a statistically significant difference ($P < 0.05$)

EB. The incorporation of nanoparticles resulted in a more efficient strategy for decreasing water solubility and WVP leading to reductions of 74.3% and 22.7%, respectively, which was attributed to the compact structure of the chitosan nanoparticles that reduced the free volume of the polymer matrix more than bulk chitosan by obstructing the diffusion of water and thereby decreasing the moisture content of the films. However, tara gum films with bulk chitosan exhibited better antimicrobial activity, as shown in Table 2.1. Authors indicated that this behavior could be attributed to the size of the CSNPs that possible is not small enough to accumulate in the bacterial membrane; besides, when the CSNPs concentration increased, the particle size increased even further due to aggregation during the film formation. Moreover, since the formation of CSNPs is based on ionic gelation mechanisms the surface charge of CS was reduced thus making it less effective against Gram-negative bacteria (*Escherichia coli*).

Pereda et al. (2014) prepared edible composite films by casting film-forming emulsions based on chitosan/glycerol/olive oil containing dispersed cellulose nanocrystals (CNs). Due to cellulose–glycerol–chitosan interactions, these complex composite films appeared less opaque as the cellulose concentration increases (up to 7 wt% CN), which balanced the reduction of film transparency due to lipid addition. Moreover, both, nanocellulose and olive oil addition led to the reduction of the water vapor permeation and the total soluble matter of the films in a concentration-dependent manner, improving at the same time their tensile behavior. Results from dynamic mechanical tests revealed that all films present two main relaxations that could be ascribed to the glycerol- and chitosan-rich phases, respectively.

As an example of multilayers edible films Basiak et al. (2016) developed composites aimed to reduce the hygroscopic character of biodegradable starch-based films. In this case rapeseed oil was incorporated by lamination, leading to samples composed by 3-layers (starch-oil-starch). According to the authors, the lipid lamination followed by starch solution casting step induced an emulsion type structure of dried films. Thus, composite films resulted more opalescent and glossier, presenting also lower TS than fatty free starch films. On the other hand, lipid incorporation

reduced the moisture absorption, particularly at higher RH, as well as the surface swelling index and the water vapor and oxygen permeability.

2.4 Active and Bioactive Edible Films

In addition, edible films could act as carriers not only of health-related compounds such as vitamins, minerals, nutraceuticals and other bioactive compounds such as colorants, flavor compounds or antibrowning agents but also of antimicrobial, antioxidant, and antisoftening agents (Raybaudi-Massilia et al. 2016; Fortunati 2016; Mellinas et al. 2016; Gutiérrez 2017; Harnkarnsujarit 2017; Moghimi et al. 2017; López-Córdoba et al. 2017). These substances can be naturally or synthetically derived, although consumers generally prefer those materials from natural sources (López-Córdoba et al. 2017; Moghimi et al. 2017). The antimicrobials extend product shelf life and reduce the risk of pathogen growth on food surfaces leading to safer food consumption (Gutiérrez 2018). The incorporation of antimicrobials to the films directly in contact to food surface, where the microbial growth and contamination are mostly found, can replace the addition of preservatives into food products (Harnkarnsujarit 2017; Nisar et al. 2017). In this line Xiao et al. (2015) also indicated that bioactive agents may not be applied directly to a food system due to possible evaporation losses, inactivation or rapid release into the food matrix. According to Nisar et al. (2017) the film approach can be more effective than applying antimicrobial substances directly to the food due to providing continuous migration of active substances into the food and thus remaining at high concentration for prolonged periods of time. Moreover, the inhibitory agents incorporated to a formulation can be specifically targeted to post-processing contaminants on the food surface (Raybaudi-Massilia et al. 2016). Diffusion of antimicrobials through an edible film or coating is influenced by the composition and manufacturing procedure of the film and coating, factors related to the food (pH, water activity), hydrophilic/hydrophobic balance of the compound, and storage conditions. Furthermore, the diffusion rates of the active compound into the product can be partially controlled by the compounds found in the coating matrix (Raybaudi-Massilia et al. 2016). All these factors need to be taken into account in order to design effective edible coating systems (Álvarez et al. 2017). Some examples of active edible films developed in the recent years are summarized below.

López et al. (2017) developed edible films with high antioxidant capacity based on salmon gelatin incorporating boldine, which is the major alkaloid obtained from Boldo tree. The concentration of both components was optimized by applying a Box-Behnken experimental design with the goal of maximizing radical scavenging capacity of film forming suspensions. Results showed synergistic effect between gelatin and boldine for both the antioxidant capacity and antimicrobial activity of gelatin against *E. coli* and *Listeria monocytogenes*. Boldine was released from the films into food simulant following the Fickian model, at a faster rate as lower was the concentration of gelatine in the film forming solution, which suggests that it is

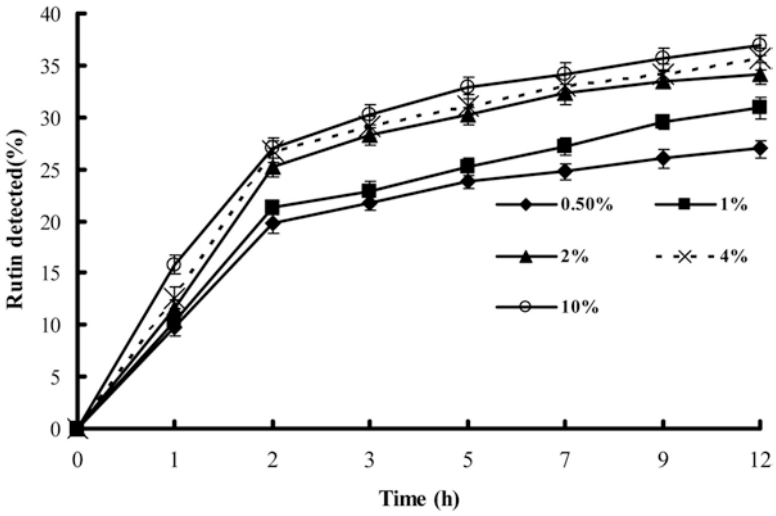

Fig. 2.3 Cumulative rutin release (%) as function of time in Milli-Q water. Samples studied were zein–rutin composite nanoparticle/corn starch films with zein–rutin composite nanoparticle contents of 0.5, 1, 2, 4, and 10% w/w. From Zhang and Zhao (2017), with permission

possible to design films to satisfy specific release criteria in order to have an active concentration of boldine in food with different shelf lives. The development of antioxidant-loaded edible films for preventing oxidation of lipid-containing foods has attracted also the attention of Zhang and Zhao (2017). These authors prepared edible active films based on zein–rutin composite nanoparticles (RNs) and corn starch. RNs (0, 0.5, 1, 2, 4, and 10%, w/w) were incorporated into the starch matrix, to act as a natural antioxidant. The RNs acted as a strong antioxidant in the corn starch films, as determined from three complementary radical-scavenging assays (ABTS, DPPH, and phosphomolybde-num), and thus the related values increased as the nanoparticle content increased. The active films also showed long-lasting antioxidant activity, which is important for the controlled release of rutin, as illustrated in Fig. 2.3. Moreover, the incorporation of RNs into the starch matrix led to the formation of a net-like structure, which decreased slightly the WVP and water solubility of nanocomposite films respect to the control film, while TS and EB increased from 1.19 to 2.42 MPa, and from 42.10 to 78.84%, respectively, with increasing RN loading. Thus, the incorporation of these complex nanoparticles into the edible films not only provided them with antioxidant properties but also improved their physical and mechanical properties.

Similar results, i.e. synergic effect of antioxidants on water barrier and mechanical properties were found by Benbettaïeb et al. (2016) for tyrosol and ferulic acid encapsulated in chitosan–gelatin films, even when this system is more complex than the previous one (i.e. a blend of polymers instead of a single polymer matrix). Moreover, these authors proved that applying radiation to the films, the retention and diffusivity of the antioxidants can be modulated. Piñeros-Hernandez et al.

(2017) incorporated polyphenols-rich rosemary extracts (RE) within cassava starch films in order to produce active food packaging with antioxidant properties. As the polyphenols content increased, the films showed an increase in their antioxidant activity. Moreover, the films containing the higher extract concentration (13.6 mg of gallic acid equivalents per gram) showed better barrier properties against UV light. However, in this case the WVP of the films increased and the elongation decreased as the extract concentration increased, which was explained considering that the presence of RE inhibited the bonding between glycerol and starch molecules. These authors also carried out migration tests using water and ethanol (95%) as food simulants for aqueous and fatty foods, respectively, finding that total polyphenols content loaded in the films migrated within the aqueous food simulant after 7 days of film exposition, while, only a negligible polyphenol amount was detected in the fatty food one.

Regarding antimicrobial active films, Arrieta et al. (2014) studied the effect of adding carvacrol into sodium caseinate (SC) and calcium caseinate (CC) matrices plasticized with two different glycerol concentrations (25 and 35 wt%). All films exhibited good performance in terms of optical properties, showing high transparency. Besides, the antimicrobial activity of SC and CC films containing carvacrol was clearly demonstrated against two indicator bacteria, *E. coli* (Gram negative) and *S. aureus* (Gram positive). Barrier properties to oxygen were excellent but diffusion of dyes through films was dependent on the caseinate type (SC or CC), since CC resulted in less permeable composites due the ability to promote cross-linking.

On the other hand, in terms of active ingredients that can be incorporated into films and coatings, essential oils (EOs) have received much attention in the last years (Calo et al. 2015; Vergis et al. 2015; Galus and Kadzińska 2015; Yuan et al. 2016; Gutierrez-Pacheco et al. 2016; Moghimi et al. 2017) due to their antimicrobial/antifungal activity and volatile nature, which facilitates the use of small concentrations that are safe for consumption (Sivakumar and Bautista-Banos 2014; Yuan et al. 2016). As the direct addition of essential oils to food may adversely impact sensory perception of applied foods, added to the fact that the essential oils lose effectiveness over time, the incorporation of these compounds into the formulation of edible films and coatings has been suggested as a better option (Yuan et al. 2016; Gutierrez-Pacheco et al. 2016). In this sense, antimicrobial edible films and coatings may provide increased inhibitory effects against spoilage and pathogenic bacteria by maintaining effective concentrations of the active compounds on the food surfaces (Gutierrez-Pacheco et al. 2016). The following are examples of recent developments in this line. Hashemi and Mousavi Khaneghah (2017) developed basil seed gum (BSG) edible films containing oregano essential oil (OEO) (1–6%). They found that WVP significantly decreased by the incorporation of OEO while moisture content, contact angle, transparency and swelling index of edible films increased. Films containing 2–6% OEO presented a significant antibacterial activity against *E. coli, S. Typhimurium, P. aeruginosa, S. aureus and B. cereus*. Moreover, the antioxidant activity (i.e. DPPH and ABTS radical scavenging activities and ferric reducing ability) of BSG films were enhanced considerably with increasing OEO concentration, as exemplified in Fig. 2.4. According to these results, the

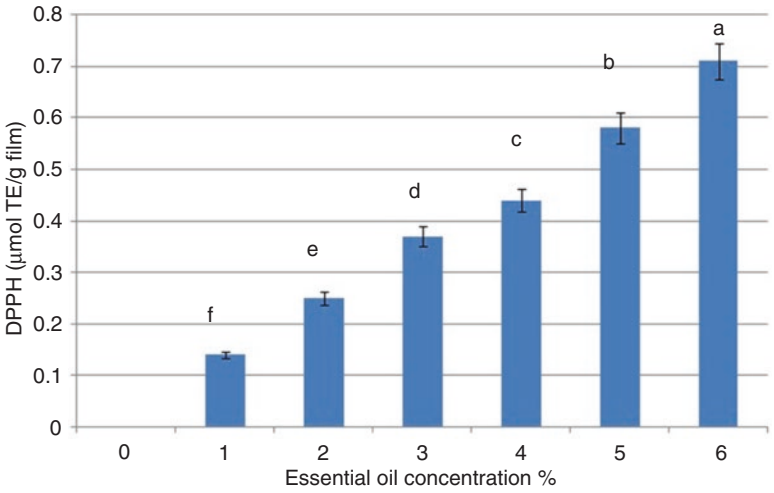

Fig. 2.4 DPPH radical scavenging activity of film at different essential oil concentration. Values represent means ± standard deviations. Each column with the same lowercase letters is not significantly different at $P < 0.05$. From Hashemi and Mousavi Khaneghah (2017), with permission

fabricated edible films with BSG and OEO can be considered for further approaching as an edible food packaging.

Moghimi et al. (2017), taking into account that EOs have low water solubility and could denature under light and heat condition, chose the nanoemulsion path for preparing antibacterial edible films based on hydroxypropyl methyl cellulose (HPMC) and Thymus daenensis (wild and cultivated) essential oil (TD EO). The authors showed the uniform incorporation of nanoemulsions into the edible film, but also that active edible films had less TS and Young's modulus compared to the control film, which was attributed to the plasticizing effect of the essential oil. However, both films exhibited a potent but differentiated antibacterial and antifungal activity, which was attributed to the variations of TD EOs components (i.e. wild plant had 53.28% thymol, while cultivated had 75.69%; the second main component of the cultivated *T. daenensis* was carvacrol (22.72%), whereas the wild plant had 25.26% of p-cymene (precursor of carvacrol)). Thus, films modified with EO extracted from the wild plant had more antibacterial activity against gram positive bacteria (including *S. aureus, S. epidermidis, B. subtilis, E. faecalis, E. faecium* and Methicillin resistant *S. aureus*) than the films based on cultivated TD. However, the formulation based on cultivated TD was more efficient against *Candida albicans* and gram negative bacteria (including *E. coli, S. typhi, S. dysenetriae, S. flexneri, A. baumannii and K. peneumoniae*). Therefore, both nanoemulsion based HPMC films could be used based on the microorganisms of interest for each packaging material.

Giteru et al. (2017) combined the previous approaches by incorporating both, citral, an antimicrobial monoterpene aldehyde extracted from the plant *Cymbopogon citratusis* and quercetin, an antioxidant compound naturally occurring in plants, into kafirin-based films. They compared the behavior of kafirin films consisting of 2.5%

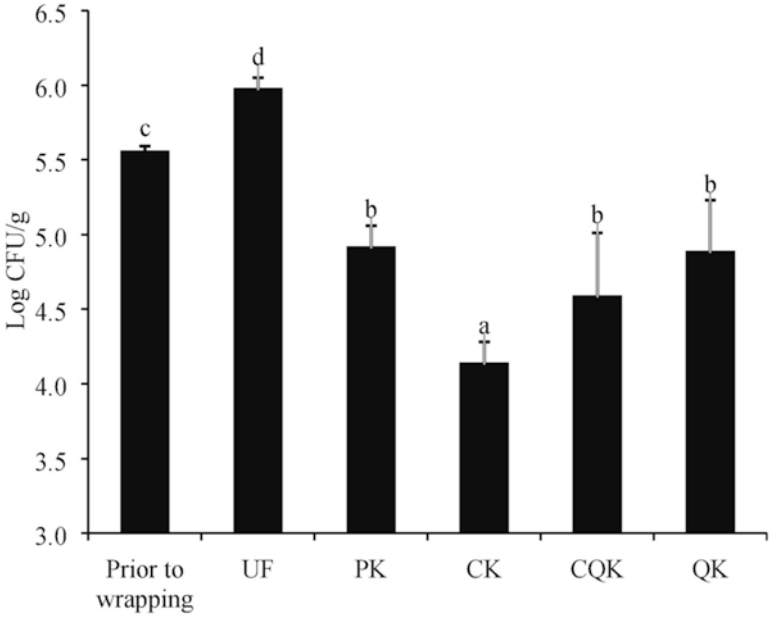

Fig. 2.5 Effect of kafirin films on the total viable count of chicken fillets stored at 2 ± 0.5 °C for 96 h. (UF) unwrapped fillet, (PK) fillets wrapped with plain film, (CK) fillet wrapped with citral film, (CQK) fillets wrapped with citral-quercetin film, (QK) fillets wrapped with quercetin film. Error bars represent ± SD (n = 9). Different letters indicate significantly different ($p < 0.05$). From Giteru et al. (2017), with permission

citral (CK), 2% quercetin (QK) or 1.25% citral +1% quercetin (CQK). It was found that incorporation of citral reduced the maximum stress and stiffness while imparting more flexibility to the kafirin bioactive films. Compared to the control kafirin (PK) and quercetin-containing films, those containing citral showed significant antimicrobial activity against the total viable count on chicken fillets stored at 2 ± 0.5 °C for 96 h, as shown in Fig. 2.5. The ability of bioactive kafirin films to inhibit oxidation of lipids on chicken fillets, which was evaluated by the thiobarbituric acid reactive substances assay, were lower for PK, CQK, and QK (0.18, 0.16 and 0.23 mg MDA/kg respectively), compared to unwrapped fillets and CK (0.41 and 0.59 mg MDA/kg respectively). These findings implied that quercetin and those polyphenolics likely co-extracted with kafirin possessed the ability to inhibit the development of lipid oxidative products. Regarding product appearance, authors found that wrapped fillets become more yellowish after the storage than unwrapped ones. This color transformation toward yellowness was attributed to the presence of citral and quercetin in the films since film swelling due to absorption of water by the hydroxyl groups in the film matrix could cause the release of originally encapsulated pigments, as indicated by Shojaee-Aliabadi et al. (2014). Thus, authors pointed out that those pigment-rich additives may have an influence on the organoleptic property of food products and that as these changes occurred on raw meat, their persistence during cooking still requires determination. Moreover, they emphasized that it is

important to determine whether this pigmentation of raw meat would be perceived negatively by consumers with further investigations.

At this point it is important to introduce the concept of bioactive packaging. The concepts of active and bioactive packaging have been sometimes used indiscriminately, but there are differences (Espitia et al. 2016). Active food packaging systems are those previously exemplified, which go beyond the traditional passive role of food protection and include desirable interactions with the food, in a way that is relevant to extend food stability; bioactive food packaging systems, on the other hand, are those which may contribute to health benefits to the consumers (Espitia et al. 2016). Bioactive packaging materials would thus hold bioactive agents, which are eventually released into the food product (Lopez-Rubio et al. 2006). In the specific case of edible bioactive films and coatings, this release is not even required, since the film/coating itself is supposed to be eaten with the food (Espitia et al. 2016). Edible films enriched with probiotics (i.e. bacteria with beneficial effects on humans and animals) are the typical examples of these bioactive films. For probiotics to play the intended role in human health, it is essential that both viability and the metabolic activity are maintained throughout food processing and supply chain, as well as within human gastrointestinal tract (Espitia et al. 2016). Several methods have been tested to increase the quality of probiotic cultures (Nguyen et al. 2016), however, microencapsulation is by far the most exploited approach (Espitia et al. 2016; Nguyen et al. 2016). When combined, microencapsulation and bioactive edible packaging concepts denote a promising strategy for protecting and delivering probiotic species efficiently. In this line, Soukoulis et al. (2017) evaluated the inclusion of whey protein isolate in different bio-polymers with established good film forming properties, for their ability to stabilize live probiotic organisms. Edible films based on low (LSA) and high (HSA) viscosity sodium alginate, low esterified amidated pectin (PEC), kappa-carrageenan/locust bean gum (k-CAR/LBG) and gelatine (GEL) in the presence or absence of whey protein concentrate (WPC) were then shown to be feasible carriers for the delivery of *Lactobacillus rhamnosus* GG. While losses of *L. rhamnosus* GG throughout the drying process ranged from 0.87 to 3.06 log CFU/g for the systems without WPC, they were significantly reduced to 0 to 1.17 log CFU/g in the presence of WPC. Films fabricated with k-CAR/LBG or HSA were most effective at maintaining maximal biological activity of the probiotic cells (0.167 and 0.218 log CFU day^{-1} in average) compared to films made of PEC, GEL and LSA (0.251, 0.252 and 0.268 log CFU day^{-1}, respectively), which was explained by the low glass transition temperature (Tg), and low WVP of the binary system. Supplementation of the film forming solutions with WPC resulted in an enhanced *L. rhamnosus GG* storage stability (0.279 and 0.183 log average CFU day^{-1} for systems with and without the addition of WPC respectively), being the bioprotective role of WPC associated with its ability to reduce the osmolytic cell injuries arising throughout the dehydration process and their excellent cell adhesion properties. Furthermore, probiotic films based on HSA/WPC and k-CAR/LBG/WPC blends had both acceptable mechanical and barrier properties. Overall this work shows that the development of edible films as carriers for the delivery of probiotics is a plausible strategy. As pointed out by Soukoulis et al. (2017), the maintenance of the biological activity of the probiotic cells is the gov-

erning parameter for the selection of the substrate compositional aspects, however, other technological parameters such as the mechanical and barrier properties are essential to ensure adequate processability and shelf life. López de Lacey et al. (2014) over passed the previous findings by developing a bioactive film based on agar and incorporating both, green tea extract (antimicrobial and antioxidant) and probiotic strains (*Lactobacillus paracasei L26* and *Bifidobacterium lactis B94*), which was applied on hake fillets in order to investigate the effect during chilled storage. Hake was previously inoculated with *Shewanella putrefaciens* and *Photobacterium phosphoreum* (10^3–10^4 CFU/g) to simulate a spoilage process. It was found that green tea/probiotic film led to a reduction of the spoilage indicators, particularly of H_2S-producing bacteria counts and total viable bacteria throughout the 15 days of storage period. However probiotics alone had a smaller effect over reducing these chemical spoilage indicators. Authors pointed out that the probiotic strains added to the film were able to pass to the fish, producing an increment of lactic acid bacterial counts, even in the presence of green tea extract. They concluded that films with green tea and probiotic could extend shelf-life of hake at least for a week and, at the same time, it could be a way to incorporate beneficial probiotic bacteria to the fish.

2.5 Conclusions

Edibility, biodegradability, and increased food safety are the main benefits of edible films. Their environmental friendly aspects make them alternatives in packaging systems, without the ecological costs of synthetic non-biodegradable materials. Although edible films and coatings have not been enough developed to be able to replace conventional plastic packaging totally, new interesting approaches are being tested every day. Therefore, there is still a considerable requirement for the development of new edible packaging materials to fulfill the increasing consumer demands for more natural foods which need to be packed and also to meet the environmental concerns and regulations. Future work in this area should ensure that the new bioactive and biodegradable materials help improving mechanical handling of food and lessen the migration of gases, volatiles, vapors and lipids in synergy with conventional packaging, but also that the incorporation of active substances into the film lead to the enhancement of the shelf-life of food products.

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