Chapter 41

Zein and Its Composites and Blends with Natural Active Compounds: Development of Antimicrobial Films for Food Packaging

A. Yemenicioğlu
Izmir Institute of Technology, Izmir, Turkey

41.1 INTRODUCTION

Due to the growing interest to develop the functional properties of packaging materials, and to obtain packaged foods with better shelf life and quality, active packaging that incorporates antimicrobials (antimicrobial packaging) has become one of the most promising research areas of food scientists. The application of a carefully designed antimicrobial packaging material can improve the safety of packaged foods by inhibiting pathogenic bacteria and/or prolonging shelf life by controlling spoilage flora using minimum amounts of active compounds (Appendini and Hotchkiss, 2002). The application of antimicrobial packaging on easily prepared, minimally processed fresh produce has gained a particular importance. This is because microbial outbreaks that originate from these products increase continuously (De Roever, 1998). Active packaging that contains antioxidants (antioxidant packaging) also attracts some interest to prevent lipid oxidation in food (Lee, 2013). Moreover, the addition of bioactive compounds into packaging materials has also initiated a novel packaging concept (bioactive packaging) that aims to enrich packaged food with bioactive compounds and improve products' potential health benefits (Lopez-Rubio et al., 2006).

The antimicrobial packaging concept is mainly applied by (1) the addition of antimicrobial-containing sachets or pads into food packages; (2) coating, immobilization, or direct incorporation of antimicrobials into food packaging materials, or (3) the use of packaging materials that are inherently antimicrobial (Appendini and Hotchkiss, 2002). However, the direct incorporation of antimicrobials (or sometimes antioxidants and other active substances) into packaging materials has become increasingly popular. This is because it enables a controlled release of antimicrobials onto food surfaces, the most susceptible part of food against microbial contamination and development (Ünalan et al., 2013). Antimicrobial chemicals, including organic or inorganic acids, metals, alcohols, ammonium compounds, and amines, can be incorporated into edible or plastic packaging materials (Appendini and Hotchkiss, 2002; Suppakul et al., 2003). However, health concerns of consumers and environmental problems have directed industrial interest towards using natural antimicrobial compounds (biopreservatives) in edible packaging materials (Appendini and Hotchkiss, 2002; Han, 2005). Due to technological problems, such as the denaturing effects of thermal polymer processing methods (extrusion and injection molding), the incorporation of biopreservatives into edible films that have mild film-forming conditions is more suitable than their incorporation into plastic films (Appendini and Hotchkiss, 2002; Suppakul et al., 2003; Han, 2000). Thus, edible films that contain biopreservatives have become a popular research topic within a very short time period. Different biopreservatives that have been tested for antimicrobial packaging include: antimicrobial peptides such as polylysine and bacteriocins, including nisin, pediocin and lacticin; antimicrobial enzymes such as lysozyme, lactoperoxidase, chitinase, and glucose oxidase; and plant extracts and essential oils that are rich in bioactive phenolic compounds (Appendini and Hotchkiss, 2002; Joerger, 2007; Labuza and Breene, 1989; Suppakul et al., 2003; Ünalan et al., 2011). Additionally, different natural biomaterials that have been frequently used in development of active edible packaging films are polysaccharides such as chitosan, cellulose derivatives, carrageenan, pectin, and alginate; and proteins such as gelatin, zein, whey, and soy (Cha et al., 2002; Han, 2000; Mecitoglu et al., 2006; Quintavalla and Vicini, 2002). In this chapter the importance of zein protein as a film-forming material and the potential of its composites and blends for use in antimicrobial

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packaging are evaluated. A particular emphasis was placed on the importance of using compatible antimicrobial agents with the zein film system. The chapter also emphasizes setting controlled release properties of zein films by exploiting the ability of zein to form blends and composites with other natural materials.

41.2 MAJOR PROPERTIES OF ZEIN AND ITS EDIBLE FILMS

Zein is a water insoluble hydrophobic storage protein found in corn and maize. It is the major coproduct of the growing oil and bioethanol industries (Manley and Evans, 1943; Selling et al., 2008; Shukla and Cheryan, 2001; Wang et al., 2007). In the food industry, zein is used mainly as a coating material for candies, fresh and dried fruits, and nuts; it is also used as an ingredient in chewing gum production (Bai et al., 2003; Lai and Padua, 1997; Shukla and Cheryan, 2001). Due to its outstanding film-forming and gas and moisture barrier properties, as well as solubility in organic solvents like ethanol, zein is attracting a growing interest as a biopolymer. Zein could be applied very easily as food coating. Alternatively, its pre-cast film could be used to wrap foods, or it could be placed on food surfaces or between food layers (Herald et al., 1996; Janes et al., 2002; Ünalan et al., 2013; Figure 1). The pre-cast zein films have also been tested successfully for the modified atmosphere packaging of vegetables (Rakotonirainy et al., 2001). In the literature, to obtain antimicrobial coatings and pre-cast films, different biopreservatives including lysozyme, polylysine, and nisin have been extensively tested in zein film systems (Dawson et al., 2000; Gucbilmez et al., 2007; Hoffman et al., 2001; Janes et al., 2002; Mecitoglu et al., 2006; Padgett et al., 1998; Teerakarn et al., 2002; Ünalan et al., 2011). The possibility of producing heat pressed zein films (Dawson et al., 2003; Padgett et al., 1998) and using thermal extrusion methods (Selling, 2010) to obtain zein films has also been studied. However, these methods are not compatible with most biopreservatives, which lose their activity during thermal processing.

The application of zein as a coating material is a quite practical process, because zein film making solutions, prepared in ethanol, could easily be applied on food surfaces by spraying, brushing, or dipping. However, the mass production of active zein-based, self-standing packaging materials is a challenging process due to the brittleness and flexibility problems of zein films. The characteristic film structure of zein consists of a meshwork that is composed of donut structures formed by asymmetric rods joined to each other (Guo et al., 2005). It is the hydrophobic interaction that keep the zein rods together and maintain film integrity (Guo et al., 2005). However, these interactions are also responsible for the brittleness and lack of flexibility in zein films. Thus, to plasticize and improve their flexibility and mechanical properties, different ingredients have been incorporated into zein films, including organic acids, sugars, alcohols, fatty acids, synthetic polymers, cross-linkers, and plasticizers (Ghanbarzadeh et al., 2006; Kim et al., 2004; Lai and Padua, 1997; Lawton, 2004; Sessa et al., 2008; Woods et al., 2009). None of these ingredients provided an effective or applicable solution to the flexibility and brittleness problems of zein films. However, Arcan and Yemenicioğlu (2011) recently obtained promising results by plastisizing zein films with different phenolic compounds. The use of natural phenolic plastisizers not only eliminates the brittleness problem of zein films (Figure 2a and b), but also increases the antioxidant and antimicrobial potential of these films (Ünalan et al., 2013).

41.3 BASIC PRINCIPLES OF DEVELOPING ANTIMICROBIAL ZEIN FILMS

Because antimicrobial packaging is a rapidly developing packaging technology, the methodology and principles of antimicrobial film development in different studies show some variation. For example, there are many studies that are "film

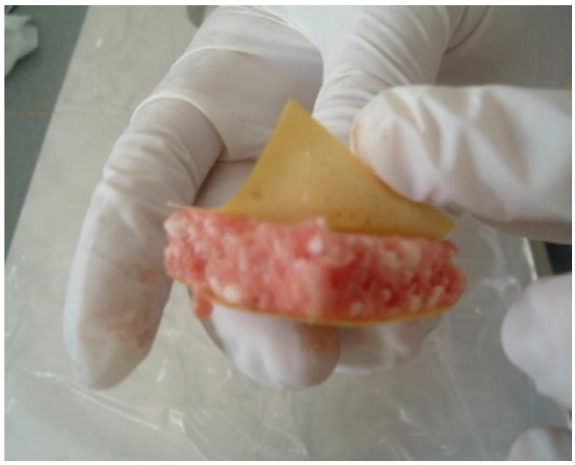

FIGURE 1 An experimental active packaging trial conducted by placing pre-cast lysozyme-containing zein films on both sides of burger pieces.

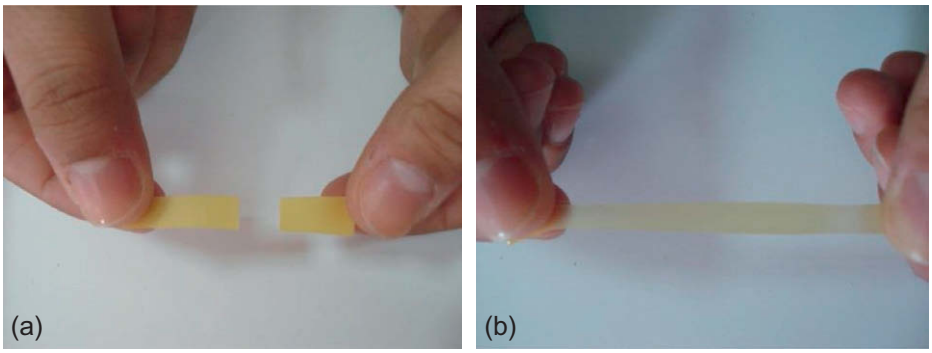

FIGURE 2 Solution of classic brittleness problem of a typical zein film with natural phenolic plastisizers. (a) Control film brakes easily by pulling; (b) gallic acid-containing film shows elongation over 100% by pulling.

material dependent" (FMD), which means that they aim to evaluate a certain problematic waste material or a low value byproduct that is rich in a film making biopolymer. In such studies the extracted biopolymer is employed in edible film making. Then, selected antimicrobial agent(s) are incorporated at different concentrations and the film is tested in laboratory media on different microorganisms. The most potent films are then further evaluated on different food by microbiological studies to determine a potential food application. The FMD type studies have some great limitations, because the fixed film system might be incompatible with some major biopreservatives and food systems. Moreover, the physical and mechanical properties of a predetermined biopolymer might be problematic, and it could be challenging to overcome these problems with the classic edible film making ingredients like plastisizers, cross-linkers, emulsifiers, anti-sticking agents, or thickening agents.

On the other hand, in some other studies the film development is more methodical and "food system dependent" (FSD). FSD type studies aim to improve the safety of a specific food product (e.g., beef burger, hamburger, or smoked salmon) against a specific target microorganism, usually pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Salmonella typhimurium,* or *Campylobacter jejuni* (Alkan et al., 2011; Ünalan et al., 2011, 2013). Some specific spoilage fungi like *Botrytis cinerea*, observed on table grapes (Zoffoli et al., 1999), or *Penicillium commune,* observed on cheese (Kure et al., 2001) might also be targeted less frequently to prevent microbial origin economic losses. Thus, in FSD type studies the selection of a suitable biopreservative(s) that is effective on the target microorganism is essential. Then comes the selection of a suitable biopolymer that is compatible with the food and the selected biopreservative. Zein film making was initiated by FMD type studies. However, the excellent film-forming capacity and high compatibility of zein with different biopreservatives have made this biopolymer one of the most suitable materials for FSD type studies.

41.3.1 Compatibility of Zein with Different Natural Antimicrobial Compounds

The main advantage of working with zein comes from its compatibility with different natural antimicrobial compounds that are mostly classified as biopreservatives. This is because of the hydrophobicity of zein, which aids in the formation of an inert film matrix that does not interact with the incorporated biopreservatives (which are mostly hydrophilic). This helps maintaining the solubility and activity of the incorporated biopreservatives in the film. It also enables the use of zein as a reservoir for different biopreservatives to achieve critical microbial inhibitory concentrations at the food surface easily. The zein film matrix could accommodate different biopreservatives including those that have protein (e.g., antimicrobial enzymes) and polypeptide (e.g., bacteriocins and polylysine) structures and those that have phenolic structures (phenolic extracts and essential oils). Some of the biopreservatives that are highly compatible with the zein film system are introduced in the following sections, along with specific comments and key points concerning their potential food packaging applications.

41.3.1.1 Lysozyme as an Antimicrobial Zein Film Component

Lysozyme, obtained from hen egg white, is the antimicrobial candidate with the most potential for use in active zein packaging. The extreme stability of this GRAS status agent, both in ethanolic zein film making solutions and in dried pre-cast zein films kept under refrigeration, has been reported by Mecitoglu et al. (2006). These authors reported that ethanol that is used in preparation of zein films causes the activation of the enzyme and increases its activity up to threefold. The same authors also reported a 100% recovery of enzyme activity in dried pre-cast zein films cold-stored for 4 months at +4 °C.

The lysozyme is effective mainly on Gram-positive bacteria and shows its antimicrobial activity by splitting the bonds between the *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan (PG) layer in the bacterial cell wall. However, it is wrong to assume that the lysozyme could be employed against all Gram-positive bacteria. For example, an antimicrobial packaging that employs lysozyme could not provide any protection against *Staphylococcus aureus* that shows an extreme resistance against this enzyme. The *S. aureus* is not only resistant against lysozyme action, but it is also capable of boosting its capacity to produce protective biofilm formation in the presence of this enzyme (Sudağidan and Yemenicioğlu, 2012).

However, the lysozyme is effective on a very critical pathogen, *L. monocytogenes* (Duan et al., 2007; Min et al., 2005; Ünalan et al., 2011). Thus, its current antimicrobial film applications are concentrated mainly on fighting against listeriosis. In fact, recent findings of Ünalan et al. (2013), who employed lysozyme-containing zein films and zein-based composite films, showed a great potential of such films to suppress the growth of *L. monocytogenes* in fresh cheeses. *L. monocytogenes* is a very critical food pathogen for dairy products, due to its incidence in raw milk and its capacity to grow at refrigeration temperatures (Kozak et al., 1996). Although fresh cheeses are mainly produced by using pasteurized milk, several listeriosis outbreaks, associated with the consumption of cheeses made from pasteurized milk, have been reported due to improper pasteurization or post-contamination (de Castro et al., 2012; Jackson et al., 2011; Johnsen et al., 2010; Yde et al., 2012). Thus, the use of lysozyme in active packaging of risky cheeses is attracting an increasing interest. Duan et al. (2007) employed lysozyme in the active packaging of Mozzarella cheese. They used chitosan films and obtained antimicrobial activity not only against *L. monocytogenes,* but also *Pseudomonas fluorescens* and *E. coli.* They reported that they obtained a 0.32-1.50 log reduction in the *L. monocytogenes* population of actively packed cheese samples. The lysozyme-containing active packaging can also be employed in risky sea products to prevent risk of listeriosis. For example, Min et al. (2005) used lysozyme in whey protein isolate films and coatings to inhibit the growth of *L. monocytogenes* in cold-smoked salmon.

All these works successfully demonstrate the suitability of using lysozyme in a wide range of packaging materials as an antilisterial agent. On the other hand, due to the protective lipopolysaccharide (LPS) layer around their PG layer, lysozyme is not directly effective on Gram-negative bacteria. However, the combined application of lysozyme with chelating agents like EDTA that cause the destabilization of LPS enables the use of this enzyme against major pathogenic Gram-negative bacteria including *S. typhimurium* and *E. coli O157:H7* (Ünalan et al., 2011). The combined incorporation of lysozyme in EDTA (more soluble disodium EDTA is used in the film system) was tested by Ünalan et al. (2011) for the active packaging of beef patties. They reported that such a packaging application could be helpful to suppress total coliform counts during 5 days of cold storage. Thus, the strategy of employing lysozyme alone or in combination with a chelating agent like EDTA depends on the target microorganism in the selected food system.

41.3.1.2 Peptides as an Antimicrobial Zein Film Component

Although the lysozyme is the biopreservative candidate with the highest potential for zein-based active packaging, GRAS antimicrobial peptides like nisin and polylysin have also been successfully tested in zein film systems (Padgett et al., 1998; Ünalan et al., 2011). Nisin, a well-known bacteriocin obtained from lactic acid bacteria, is a cationic peptide. It shows antimicrobial activity by interacting with the anionic phospholipids at the bacterial surfaces and forming pores and dissipating proton motive forces at the bacterial membrane (Sudağidan and Yemenicioğlu, 2012). Similar to lysozyme, nisin can not overcome the protective LPS of Gram-negative bacteria, and shows antimicrobial activity mainly on Gram-positive bacteria. However, the advantage of using nisin in active packaging comes from its potency against *S. aureus,* which is quite resistant to the action of lysozyme. In a recent study, Sudağidan and Yemenicioğlu (2012) showed that the presence of 25 μg/ml nisin is sufficient to inactivate all of the 25 *S. aureus* strains isolated from raw milk and cheese samples. The food poisoning caused by *S. aureus* is frequently associated with raw milk and traditional cheeses made from raw milk, because the breasts of dairy cows are often contaminated with enterotoxigenic strains of *S. aureus* that cause mastitis (Oliver et al., 2005; Pinto et al., 2011). Due to its ability to produce biofilms formed by an extracellular polysaccharide matrix and biofilm associated proteins (Cucarella et al., 2004), it is exceptionally difficult to control risks caused by *S. aureus*. The biofilm formed by the bacteria increases its resistance to mechanical cleaning and disinfectants, and this causes it to spread to different parts of processing environments and easily contaminate food. Thus, even cheese obtained from heat treated milk and whey might also have significant risk of *S. aureus* contamination, unless processing equipment is decontaminated effectively and the curd that is obtained post-heating is handled and stored properly (Jakobsen et al., 2011).

The active packaging conducted by zein films that incorporate nisin could be employed as an important part of a hurdle to reduce risks associated not only from cheese obtained from heated milk, but also from traditional chesses which are still produced locally from unheated milk. The same problem also exists for *L. monoctogenes,* another well-known biofilm former, which can also colonize on the breasts of dairy cows and cause contamination of milk (Borucki et al., 2003;

Hunt et al., 2012). However, the use of nisin-containing active packaging against *L. monoctogenes* should be evaluated as one of the legs of a hurdle concept, because there are reports about possible nisin adaptation of this deadly bacterium (Harris et al., 1991). One of the options to overcome bacterial resistance problems is the combination of nisin with lysozyme. It has been reported that the combinational application of nisin and lysozyme shows synergy against Gram-positive bacteria, including pathogenic ones like *S. aureus* (Chung and Hancock, 2000; Sobrino-López and Martin-Belloso, 2008). Gill and Holley (2000) also employed this strategy in bologna sausages and reduced the growth of *L. monocytogenes* during a 2 week period. The combinational application of nisin and lysozyme also gave promising results against *L. monocytogenes* in ready-to-eat seafood products (minced tuna and salmon roe) (Takahashi et al., 2012) and in ready-to-eat turkey bologna (Mangalassary et al., 2008). Both nisin and lysozyme are highly compatible with the zein film system, thus, combinational incorporation of nisin and lysozyme into zein films might be an alternative option to improve effectiveness of antimicrobial zein packaging.

Another natural antimicrobial peptide that is highly compatible with the zein film system is ϵ-polylysine. ϵ-polylysine is formed by 25-35 L-lysine residues, and it is produced commercially from aerobic fermentation by *Streptomyces albulus*, which is a nonpathogenic microorganism (FDA, 2004; Geornaras and Sofos, 2005; Hiraki et al., 2003; Ting et al., 1999). The major advantage of using ϵ-polylysine as an antimicrobial agent is that it is effective on major Gram-positive and Gram-negative food pathogenic bacteria such as *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella typhimurium* (Geornaras and Sofos, 2005; Geornaras et al., 2007). The antibacterial action of ϵ-polylysine is attributed to its polycationic and surface active nature that enables its interaction with bacterial membranes (Ho et al., 2000). However, a recent study by Liu et al. (2015) suggested that the ϵ-polylysine is also capable of interacting with bacterial DNA when it penetrates into cells. Liu et al. (2015) also showed that the combination of ϵ-polylysine with nisin creates a synergetic antimicrobial activity against *E. coli* and *S. aureus*. In Japan, ϵ-polylysine has been approved for use in sliced fish and fish surimi, boiled rice, noodle soup stocks, noodles, and cooked vegetables (Hiraki et al., 2003), while the US FDA recognized ϵ-polylysine as safe (GRAS) for use in cooked or sushi rice (FDA, 2004). However, recent report of Chang et al. (2010) suggested that ϵ-polylysine could also be used in meat products against critical pathogenic bacteria, such as *L. monocytogenes*, *E. coli* O157:H7, and *S. typhimurium*. The high compatibility of ϵ-polylysine with zein is demonstrated by Ünalan et al. (2011), who also tested this natural antimicrobial in chitosan, whey, and alginate film systems. These researchers attributed the good antimicrobial potential of ϵ-polylysine in the zein film system to its hydrophobic nature, and the limited charged groups of zein that prevent the complexation and immobilization of ϵ-polylysine with the film matrix by charge-charge interactions (Ünalan et al., 2011).

41.3.1.3 Phenolic Compounds as an Antimicrobial Zein Film Component

Phenolic compounds are among the most potent and abundant natural antimicrobial compounds. They can be obtained from plant-based agro-industrial waste and byproducts. The antimicrobial activity of phenolic compounds may occur by multiple mechanisms, including complex formation with cell walls, membrane disruption, inhibition of bacterial adhesion, or inactivation of bacterial enzyme systems (Cowan, 1999). The use of natural phenolic compounds in food packaging is particularly encouraged since they also improve the antioxidant quality of food and show different benefits on human health (Lopez-Rubio et al., 2006; Wang et al., 2012). Zein provides an excellent opportunity to use pure phenolic compounds, phenolic extracts, and phenolic rich essential oils in active packaging, because its films are prepared in ethanol, an effective solvent for most phenolic compounds. The molecular weight and structure of phenolic compounds can show great variation, and they may contain different numbers of hydroxyl groups, which are capable of forming H-bonds with peptide carbonyl groups of proteins (Damodaran, 1996). Thus, the interaction and bonding of phenolic compounds into a zein film matrix might cause some different changes in structure, antimicrobial activity, and mechanical properties of the films. Arcan and Yemenicioğlu (2011) recently showed that the incorporation of phenolic compounds offers a new avenue for the use of zein films as flexible bioactive packaging materials. They reported that the phenolic compounds, particularly hydroxyl cinamic acids like gallic acid and ferulic acid, hydroxybenzoic acids like *p*-hydroxy benzoic acid, and flavonoids like (+) catechin, act as natural plastisizers for the zein film matrix (Arcan and Yemenicioğlu, 2011). It seems that the mechanism of the plastisization of zein by phenolic compounds is similar with that of most plastisizers and related with their hydroxyl groups, which form hydrogen bonds with the biopolymer to increase the free volume of the film matrix (Sothornvit and Krochta, 2005). The hydrophilic hydroxyl groups of phenolic compounds also decrease the hydrophobic interaction among zein molecules. This contributes to their increased mobility and flexibility (Alkan et al., 2011; Arcan and Yemenicioğlu, 2011). On the other hand, the effects of phenolic compounds on structural properties of zein films show great variation. For example, catechin reduces the zein film porosity, while gallic acid reduces pore size, but increases the number of pores, and *p*-hydroxy benzoic acid and ferulic acid mainly increase pore size (Arcan and Yemenicioğlu, 2011). These changes in film

morphology gain a particular importance when the controlled release of the active substance is a critical factor to obtain a benefit from active packaging.

The use of catechins in foods as a bioactive ingredient has been continuously increasing since these compounds are well characterized for their health benefits and are already involved in human diet in large amounts by consumption of green and black tea, chocolate, red wine, and fresh fruits and vegetables (Yilmaz, 2006). The major catechins include catechin, epicatechin, epicatechin gallate, and epigallocatechin gallate (Yilmaz, 2006). However, the catechin is one of the most potential candidates for active zein packaging since it has already been tested in zein films (Arcan and Yemenicioğlu, 2011, 2014; Ünalan et al., 2013) and in several different edible film systems (Ku et al., 2008a,b) to exploit its antimicrobial and antioxidant properties. Other potential phenolic alternatives in development of antimicrobial zein films are phenolic extracts. The use of grape seed extract and green tea extract in edible films attracts a particular interest since foods containing these popular extracts are particularly demanded by the consumers (Perumalla and Hettiarachchy, 2011). The catechins are the main active phenolic compounds in green tea extracts and up to 50% of the catechins in the green tea is formed by epigallocatechin gallate that might show antimicrobial effect on both Gram-positive (*Staphylococcus* spp.) and Gram-negative (*Salmonella* spp.) bacteria (Perumalla and Hettiarachchy, 2011). On the other hand, the grape seed extracts are formed mainly by the oligomeric proanthocyanidins that have a red color and astringent taste which might affect the sensory properties of food (Perumalla and Hettiarachchy, 2011). Besides phenolic extracts essential oils such as thymol, carvacrol, eugenol, and citral are also extensively tested in edible film systems as antimicrobial and antioxidant agents. According to FDA thymol and carvacrol are considered as food additives, thus, they have lower potential than eugenol and citral which are in GRAS status (Suppakul et al., 2003). However, thymol has been extensively tested in zein film system and its antimicrobial effectiveness on important pathogenic bacteria, yeast and mold and release kinetics from zein films have been studied with details (Del Nobile et al., 2008; Gutierrez et al., 2009; Mastromatteo et al., 2009). Park et al. (2012) employed eugenol, thymol, and carvacrol incorporated zein films for lamination of low-density polyethylene films intended for antioxidant packaging. Khalil and Deraz (2015) employed eugenol to develop antimicrobial zein films and to improve their mechanical properties. However, it is important to note that the essential oils have a distinctive odor and taste that is incompatible with most food systems. Thus, it was suggested that the application of essential oils in active packaging should be combined with use of suitable flavor compounds (Gutierrez et al., 2009). Gutierrez et al. (2009) found that an acceptable organoleptic profile could be obtained by combining carvacrol and thymol with vanilla aroma while strawberry aroma is compatible only with thymol and banana aroma is compatible with none of the specified essential oils tested.

41.3.2 Control of Antimicrobial Release from Zein Films

The antimicrobial packaging mainly targets the food surface on which microbiological changes occur most intensively. However, a sufficient antimicrobial effect could not be achieved unless the release rate of antimicrobial compounds from the packaging materials to the food surface is adjusted considering several complex factors. Some of these complex factors include the physical and chemical properties of food, the growth kinetics of target pathogenic or spoilage microorganisms, and the expected shelf life of the food (Appendini and Hotchkiss, 2002; Han, 2005). A very rapid release of antimicrobials from film onto food surface causes diffusion of antimicrobials through the inner parts of food and the critical food surface is left unprotected. In contrast, too slow of a release of antimicrobials from film onto food surface prevents the antimicrobials from reaching of the critical inhibitory concentration of target bacteria at the food surface. The sustained release of antimicrobials is particularly important for effectiveness of zein films, because they have a very fast release profile for most biopreservatives, including lysozyme and phenolic compounds (Arcan and Yemenicioğlu, 2014; Mecitoglu et al., 2006). Although zein is mainly a hydrophobic biopolymer, its films have a porous structure and it still has some hydrophilic and amphiphilic constituents that cause its limited swelling. This is a very important problem when zein films incorporate lysozyme, a highly hydrophilic enzyme which rapidly diffuses from films that show swelling.

In the literature, different solutions are offered to control the rapid release of lysozyme from plastic and edible films that show swelling in aqueous media. For example, to control lysozyme release rates, Buonocore et al. (2005) produced multilayered PVOH films and changed the degree of crosslinking for PVOH films. Gemili et al. (2009) developed asymmetric cellulose acetate films with varying porosities to control lysozyme release rates, while Bezemer et al. (2000) changed composition and molecular weight of copolymers in biodegradable polyethylene glycerol/poly butylenes trephthalate films. Mendes de Souza et al. (2010) worked with lysozyme-containing sodium caseinate films and achieved its controlled release by modifying the pH and the amount of crosslinking agents, such as $CaCl_2$, transglutaminase, and glyoxal, used in film making. Park et al. (2004) modified release rates of chitosan-lysozyme composite films by changing the amount of enzyme within the composite structure.

41.3.2.1 Use of Zein-Wax Composite Films

The use of composite structures in zein film making is quite beneficial to control the release rates of antimicrobial agents. The combination of zein with a more hydrophobic film making agent serves to increase film hydrophobicity and reduce film swelling in aqueous media. A composite structure could also increase film tortuosity and could help reduce an antimicrobial's diffusion coefficients (Ozdemir and Floros, 2003). The most practical and applicable method to obtain zein composite films is the incorporation of waxes, such as beeswax, carnauba wax, or candelilla wax, into these films. The addition of these waxes into ethanolic zein film making solutions at 5% (w/w) of zein produces uniform composite films. This can only be achieved if waxes are added into boiling film making solution to enable their melting, and hot film making solutions are homogenized in the presence of a suitable emulsifier like lechitin (Arcan and Yemenicioğlu, 2013; Figure 3a and b). Ünalan et al. (2013) reported that the use of zein-wax composite films is highly effective in sustaining lysozyme release rates. However, these authors also noted that the beneficial effects of composites on sustained lysozyme release become sound when films are plastisized using catechin. The catechin is not only an effective plastisizer for the zein, but it also improves sustained release properties of zein films by reducing their pore size (Arcan and Yemenicioğlu, 2013; Ünalan et al., 2013). The catechin is also a potent antioxidant compound and has been successfully tested in different active packaging systems (Ku et al., 2008a,b). In contrast, the use of low molecular weight phenolic acids like gallic, ferulic, and *p*-hydroxybenzoic acids in plastisization of zein films increases the pore size or number of pores of films and impairs their sustained antimicrobial release properties (Alkan et al., 2011; Arcan and Yemenicioğlu, 2011).

The type of wax used in composite making also affects the sustained release properties of films. Waxes that have low melting points (MP) are more easily mixed with zein and more homogenously distributed within the zein film matrix. Thus, a wax with a very low MP impairs the composite structure by reducing the amount of tiny wax particles and aggregates within the films. This causes a reduction in film tortuosity that has a major impact on the sustained release of antimicrobials. The effect of the MP of waxes on the sustained release profiles of zein-wax composites is clearly observed by Arcan and Yemenicioğlu (2013). These authors reported that the use of beeswax (MP: 62-66 °C) in zein composite film making, in place of candelilla wax (MP: 68.5-72.5 °C), causes a 1.7-fold increase in the release rate of lysozyme. However, both composite films still showed 1.8-2.5-fold lower lysozyme release rates than zein control films that lacked the composite structure (Arcan and Yemenicioğlu, 2013). Ünalan et al. (2013) tested the antimicrobial and antioxidant potential of zein and zein-wax composite films, which have different release profiles, for lysozyme and mixtures of lysozyme, catechin, and gallic acid. The films were tested on cold-stored fresh Kashar cheese that was inoculated with *L. monocytogenes*. The authors reported that all lysozyme-containing films prevented the increase of *L. monocytogenes* counts in Kashar cheese for 8 weeks at 4 °C. However, only the zein-wax composite films, with sustained lysozyme release rates, caused a significant reduction (−0.4 decimals) in the initial microbial load of the inoculated cheese samples. Ünalan et al. (2013) also reported that the incorporation of the mixture of catechin and gallic acid into zein films showed no considerable antimicrobial effect in cheese. However, the oxidative changes in cheese packed with films that contained phenolic compounds were successfully controlled.

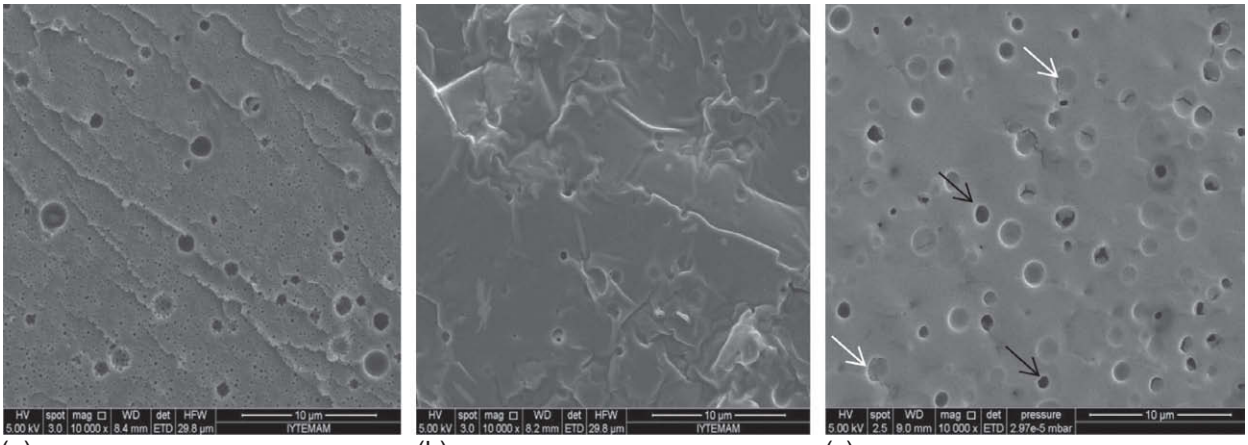

FIGURE 3 Scanning electron microscopic images (×10,000) of cross-sections of zein film. (a) Zein-carnauba wax composite film. (b) Zein-oleic acid blend film. (c) Black arrows, pores; white arrows, microspheres.

41.3.2.2 Use of Zein-Fatty Acid Blend Films

The use of blend films is an alternative method to control the release profiles of antimicrobials from edible zein films. Adding different concentrations of suitable fatty acids into zein film making solutions, with the aid of emulsifiers and homogenization, is a highly applicable method to obtain zein blend films. Arcan and Yemenicioğlu (2014) reported that the hydrophobicity and morphology of zein films can successfully be modified by blending oleic ($C_{18:1}$)Δ^9, linoleic ($C_{18:2}$)$\Delta^{9,12}$, or lauric (C_{12}) acids at 10% (w/w) of zein in the presence of the emulsifier lecithin. These authors determined that the zein blend films show 2-8.5 and 1.6-2.9-fold lower initial release rates for the model active compounds, lysozyme and catechin, than the zein control films, respectively. Moreover, they reported that the increase of fatty acid chain length reduces the release rates of active compounds considerably. The controlled release properties of zein-fatty acid blend films are attributed mainly to the microspheres formed within their film matrix and the encapsulation of active compounds (Arcan and Yemenicioğlu, 2013, 2014).

The morphological changes of zein film structure, when it is mixed with fatty acids without use of an emulsifier, were first explained by Wang et al. (2008). According to these authors the morphological changes in the zein-oleic acid film system occur in three steps: (1) formation of large numbers of oleic acid-coated zein microspheres, (2) partial melting of the microspheres by means of oleic acid, and (3) transformation of a sponge like morphology by interconnection of microspheres with channels and tunnels. Arcan and Yemenicioğlu (2013) employed the zein-oleic acid blend films for the controlled release of lysozyme, and also reported the presence of microspheres within these films. However, different from Wang et al. (2008), these authors conducted the blending process by using lecithin emulsifier, and they did not observe the presence of interconnections among the formed self-standing microspheres (Figure 3c). Thus, they proposed that the repulsion formed by negative charges of lecithin, which interacted with the oleic acid coating formed around zein microspheres, might prevent the aggregation and melting down of the microspheres. This might enable the formation of self-standing microspheres, different from the sponge-like structure observed by Wang et al. (2008). It was also noted that the addition of catechin into zein films reduces the sizes of microspheres, but causes an increase in the number of microspheres (Arcan and Yemenicioğlu, 2013; Arcan and Yemenicioğlu, 2014).

41.4 CONCLUSIONS

In this chapter the potential of zein as an edible antimicrobial film making material has been discussed with advantages and disadvantages. The major advantages of using zein in edible film making are: (1) evaluation of an important byproduct/waste from oil and bioethanol industries; (2) solubility in ethanol and simple food coating formation by spraying, brushing, and dipping; (3) high compatibility with incorporated biopreservatives that have protein, peptide, and phenolic structures; and (4) the ability to form composites and blends with lipids and fatty acids to improve sustained release properties for biopreservatives. On the other hand, major disadvantages of zein include: (1) classic brittleness problems that appear particularly during use of self-standing films; (2) highly variable prices, grades, and purity of commercial zein preparations; and (3) further studies are needed to improve commercial methods of self-standing zein film production. At this point the current technology and knowledge enables commercial application of zein-based films only in antimicrobial food coating applications. However, there is a growing interest in zein production and research to develop self-standing zein films. This valuable protein could play important roles in the future as a plastic alternative biopolymer.

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